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$_2$ receptor is implicated in autoinhibition of histamine synthesis and release and the H$_3$ receptor in gastric acid secretion, whereas the H$_3$ 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 embryonic carcinoma (EC) 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.
**EXPERIMENTAL PROCEDURES**

**Chemicals—**Histamine dihydrochloride, pyrilamine (maleate salt), leucotrienes, 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), GTP-\(\gamma\)S was from Boehringer Mannheim, okadaic acid was from Life Technologies, Inc., and [pyridinyl-5-\(\text{\textsuperscript{3H}}\)]pyrilamine ([\(\text{\textsuperscript{3H}}\)]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 DM-medium supplemented with 7.5% FCS. ESS and D3 ES cells were maintained in conditioned minimal essential medium (Life Technologies, Inc.) supplemented with 10 mM \(\beta\)-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 plates were plated to yield subconfluent cultures for experimentation.

**Ca\(_{\text{2+}}\) Determinations—**For Ca\(_{\text{2+}}\) measurements, cells were maintained in serum-free medium for 1 h and subsequently loaded with 10 \(\mu\)M Fura-2 acetoxymethyl ester for 30–45 min at 33°C in a Heps-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 \(\text{pH} 7.3\) 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 \(\mu\)M AlCl\(_3\), 5 mM KCl, 2 mM CaCl\(_2\), 10 mM Hepes, and 10 mM glucose, \(\text{pH} 7.3\). 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 fluorometer. Emission fluorescence was digitally sampled at 340 and 380 nm and corrected for background fluorescence as determined from unlabeled cells. The intracellular Ca\(_{\text{2+}}\) 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 \(\pm 51\) mV, and cells were clamped at this potential. Currents were analyzed as described earlier (19).

**\([\text{\textsuperscript{\text{3H}}}]\)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 \(\mu\)M [\(\text{\textsuperscript{3H}}}\)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 \(\mu\)M), the same buffer was used as described for Ca\(_{\text{2+}}\) determinations and applied for 20 min at room temperature, after which the cells were placed on ice and the [\(\text{\textsuperscript{3H}}}\)pyrilamine binding assay was performed. For experiments with GTP-\(\gamma\)S, 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. GTP-\(\gamma\)S was added to the permeabilized cells and incubated shortly (1–2 min) at room temperature to allow GTP-\(\gamma\)S to enter and sensitize the cells. Thereafter, cells were plated 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.

**RESULTS**

**Induction of Histamine Signal Transduction in F9 EC and D3 ES Cells—**Different EC and ES cell lines showed marked differences in their response toward histamine (1 \(\mu\)M). P19 EC cells and ESS cells responded to histamine with a marked increase in cytosolic Ca\(_{\text{2+}}\) levels and transmembrane currents, as assayed with whole-cell patch clamp electrophysiology and fluorimetric Ca\(_{\text{2+}}\) 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 ESS 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 \(\mu\)M leukotriene D\(_4\), 1 \(\mu\)M leukotriene E\(_\alpha\), or 3 \(\mu\)M serotonin induced an ability in these cells to respond to histamine: after prestimulation with one of these compounds, both histamine-induced Ca\(_{\text{2+}}\) 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 \(\mu\)M) and bradykinin (1 \(\mu\)M) but never did show induced histamine responsiveness (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**
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 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 mM) and (+)-cicletanine (15 mM) but not by the $H_1$-unspecific enantiomer (-)-cicletanine (15 mM). 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. 4B; 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

TABLE I

| 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  |
| 1 mM NaF + 75 mM AIF$_3$ + 0.1 mM Histamine | 10/20 | ND  |

TABLE II

| Condition                        | No. of responses | Histamine-induced current |
|----------------------------------|------------------|---------------------------|
| 0.1 mM histamine                 | 0/15             | pA                        |
| 5% SH-FCS + 0.1 mM histamine     | 9/9              | 90 ± 50                   |
| 1 mM LTD$_4$ + 0.1 mM histamine  | 4/4              | 70 ± 10                   |
| 3 mM serotonin + 0.1 mM histamine | 6/7          | 80 ± 50                   |
| 100 ng/ml pertussis toxin + 5% SH-FCS + 0.1 mM histamine | 0/4   | 120 ± 80                   |
| + 0.1 mM histamine               |                 |                           |
| + 100 ng/ml pertussis toxin + 1 mM LTD$_4$ + 0.1 mM histamine | 0/4 | 100 ± 80                  |

Sensitization of the Histamine $H_1$ Receptor

FIG. 3. The effect of histamine on the intracellular Ca$^{2+}$ concentration in F9 EC cells. Pseudo-color images of video-recorded Fura-2AM-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).
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. 4C, 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, leukotriene, 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. 4D).

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 \(\mu\)M GTP\(_{\gamma}\)S. This procedure was indeed sufficient for inducing histamine responsiveness, as assayed with whole-cell patch clamp (Fig. 5B), whereas GDP\(_{\beta}\)S injection did not produce this effect. The GTP\(_{\gamma}\)S-induced histamine responsiveness was, however, of a highly transient...
nature (Fig. 5C), 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 H1 receptor antagonist pyrilamine (Fig. 6) but not by cimetidine (a H2 receptor antagonist), demonstrating that this histamine responsiveness is mediated by the H1 receptor.

Treatment of cells with 20 mM NaF and 75 μM AlCl3 (which potently activates G proteins) led within 5 min after application to a slow but sustained increase in intracellular Ca2+ (Fig. 5A), probably due to activation of Ca2+-mobilizing G protein-dependent signaling elements. Importantly, such a treatment also induced histamine-dependent Ca2+ responses on top of the aforementioned sustained increase in intracellular Ca2+ levels within 20 min after application of AlF3 (Fig. 5A; Table I). We concluded 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 [3H]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 [3H]pyrilamine binding was observed (n = 7; Fig. 7A), in contrast to P19 EC cells (which react unconditionally to histamine), which exhibited high affinity binding of [3H]pyrilamine (kD ≈ 7 nM; n = 2). In accordance, whole cell membrane preparations of P19 EC cells displayed high affinity binding of [3H]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 [3H]pyrilamine binding with a Kd of 19 ± 4 nM and a Bmax 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 AlCl3 induces high affinity [3H]pyrilamine binding with an apparent Kd of 24 ± 3 nM and a Bmax of 0.31 ± 0.12 pmol/10^6 cells (n = 4; Fig. 7B). As our observations with regard to the transient nature of the induced histamine responsiveness predicted a transient induction of [3H]pyrilamine by external sensitizing stimuli like leukotrienes and AlF3, we also tested whether such transient [3H]pyrilamine binding to cells could be demonstrated. At 37 °C, these experiments yielded rather variable results, probably because only after 1 h of labeling, [3H]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 [3H]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 H1 receptor action in F9 EC cells. Therefore, these results define a hitherto undescribed mechanism controlling H1 receptor function.

**DISCUSSION**

In the present study we show that H1 receptor signaling in F9 EC cells and ES5 cells required prestimulation of the cells with either fetal calf serum, serotonin, leukotriene D4, or leukotriene E4, whereas bradykinin and ATP did not produce this effect. This sensitizing effect was inhibited by pertussis toxin, whereas it was mimicked by GTPγS and AlF3. Therefore, pertussis toxin-sensitive G proteins are probably mediating this regulation of H1 receptor action. The molecular basis for the induction of H1 receptor responsiveness appears to be the appearance of high affinity ligand binding sites, as control cells did not display high affinity [3H]pyrilamine binding, but introduction of GTPγS or treatment with AlF3 immediately provoked such high affinity [3H]pyrilamine 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 H1 receptor action.

The molecular details, however, by which external stimuli enable the H1 receptor to interact with its ligand, remain unclear. An explanation for the impaired H1 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 H1 receptors, it has been found to occur with several other G proteins.

**Fig. 5.** Induction of histamine responsiveness in F9 EC cells by AlF3 and GTPγS. A shows a representative Ca2+ tracing of the effect of 20 mM NaF and 75 μM AlCl3 on the responsiveness to histamine (HA) added 6 min later. Pretreatment with NaF and AlCl3 permitted a Ca2+ 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.

- Sensitization of the Histamine H1 Receptor

- **DISCUSSION**

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- The molecular details, however, by which external stimuli enable the H1 receptor to interact with its ligand, remain unclear. An explanation for the impaired H1 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 H1 receptors, it has been found to occur with several other G proteins.
amine binding, the amount of label nondisplaceable with 100 percentage of total unbound and bound at the same time. The bound at each time point is the total specifically bound expressed as a binding (16). 

\[ \text{specific binding} = \text{total binding} - \text{nonspecific binding} \]

with NaF and AlCl₃ (filled circles). The solid line indicates the induced high affinity binding, whereas the dotted line indicates the constitutive nonspecific low affinity binding (16).

FIG. 7. Induction of high affinity \[^3H\]pyrilamine binding sites by GTPγS (A) and AlF₄⁻ (B). A shows a Scatchard plot representing the binding of \[^3H\]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 CaCl₂, 2 mM EGTA, 0.1 mM MgCl₂, 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. Scatchard analysis and fitting was performed as described earlier (16). B, displays a Scatchard plot of \[^3H\]pyrilamine binding to unpermeabilized cells (open circles) and to unpermeabilized cells treated with NaF and AlCl₃ (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 \[^3H\]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 \[^3H\]pyrilamine by external sensitizing stimuli like leukotrienes and AlF₄⁻, without the need for cell permeabilization. We tested whether such transient \[^3H\]pyrilamine binding to intact cells could be demonstrated. Cells incubated with 4 nM \[^3H\]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 \[^3H\]pyrilamine binding, the amount of label nondisplaceable with 100 μM cold pyrilamine was subtracted. Under these conditions, transient induction of high affinity \[^3H\]pyrilamine binding becomes visible. \[^3H\]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 H₂ 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 endosomal 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 \[^3H\]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 H₁ 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 H₁ 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 H₁ 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 a₂-adrenergic receptor, where receptor phosphorylation is associated with deactivation (29). Further biochemical and mutational characterization of the H₁ receptor is required to determine whether specific phosphorylation sites are involved in the regulation of the receptor affinity and activity.

H₃ receptor activation has been implicated in processes like inflammation and anaphylaxis, and therefore H₃ receptor action must be carefully regulated. It is to be expected that mechanisms have evolved for controlling the signaling by the H₃ receptor. The regulation of histamine responsiveness as described in the present study provides such a control mechanism because for stimulation of the H₃ 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 histo-
mine reactivity may be exerted, but further studies are re-
required to assess the importance of this mechanism in these
pathological conditions. These studies are currently under
progress.

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