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Interaction of histamine with specific membrane receptors on gastric mucosal cells*

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Histamine stimulates the secretion of acid and the formation of cyclic AMP in gastric mucosa of several species [1, 2], and it increases both cyclic AMP and [^{14}C]aminopyrine uptake in isolated gastric cells from guinea pig, dog and rat [3–10]. It also stimulates adenylate cyclase activity in guinea pig gastric mucosal membranes [11]. Direct studies of histamine with biologically active H_2-receptors in gastric cells have been hindered by the lack of suitable isolated cell system. To investigate further the biological significance of histamine–receptor interaction *in vitro*, it is desirable to study simultaneously the biological response of an intact cell system to histamine under conditions wherein direct binding to the receptors on intact cells could be determined.

In the present work we used our recently developed technique for isolating gastric mucosal cells from guinea pig stomach to study the kinetics, stoichiometry, and specificity with which [^{3}H]histamine interacts with H_2-receptors on mucosal cells.

Dispersed gastric cells were prepared as previously described [12]. The cells were suspended in standard solution containing Hanks' buffer (GIBCO) plus 15 mM Hepes [4-(2-hydropyethyl)-1-piperazine-ethanesulfonic acid], pH 7.2 [12]. Binding of histamine (7–10 Ci/mmole, New England Nuclear Corp., Boston, MA) was determined by our centrifugation technique as previously described [5]. Gastric cells (2–4 × 10^6 cells) were incubated in 0.5 ml standard solution containing 0.1 to 0.25 μCi [^{3}H]histamine. The cells were washed twice with 20 vol. of iced standard solution, suspended in 1% (v/v) Triton X-100, and dispersed in Aquasol. To determine specific binding of [^{3}H]histamine, 1 mM histamine was added to parallel incubations. The cell-associated radioactivity in these incubations was subtracted from the total observed binding to obtain specific binding. Binding of [^{3}H]histamine was a linear function of cell concentration from 1 to 15 million cells/ml. Cellular cyclic AMP and uptake of [^{14}C]aminopyrine (18 mCi/mmole, New England Nuclear) were determined as described in detail elsewhere [3, 5].

Binding of [^{3}H]histamine to gastric cells at 37° was moderately rapid ($T_{\frac{1}{2}}$, 14 min) and reached steady state by 40–50 min of incubation (Fig. 1A). Adding unlabeled histamine (1 mM) to the incubation solution reduced tracer binding by 85 per cent, indicating that binding of [^{3}H]histamine occurred to a finite number of receptors which could be saturated at a high concentration of histamine. Reducing the incubation temperature from 37 to 4° decreased [^{3}H]histamine binding by 94 per cent (Fig. 1A), as seen in other histamine-receptor binding studies [13]. To examine the reversibility of the binding process, cells were incubated with [^{3}H]histamine at 37° for 60 min, washed to remove free radioactivity, and resuspended in fresh standard solution containing no radioactivity. The loss of bound [^{3}H]histamine followed a first-order process with a dissociation rate coefficient of 0.04 min^{-1} (Fig. 1B and insert). Adding 1 mM histamine to the cells did not change the rate of dissociation, suggesting the absence of

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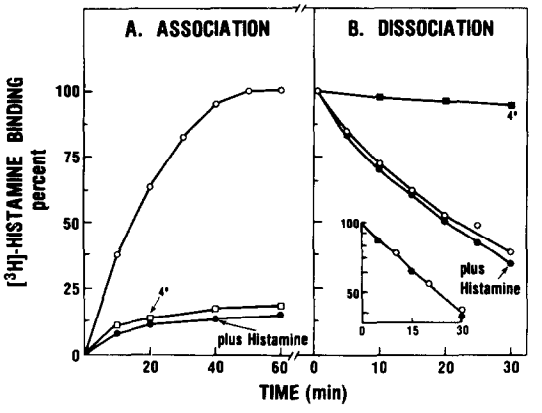

Fig. 1. Time course of [^{3}H]histamine binding to dispersed mucosal cells from guinea pig stomach. (A) *Association.* Cells were incubated with [^{3}H]histamine (concentration 20 nM) plus the indicated agents. Maximum [^{3}H]histamine binding was 0.15 ± 0.02 pmole/10^6 cells. (B) *Dissociation.* Cells were preincubated with [^{3}H]histamine for 60 min at 37° and then washed three times with, and resuspended in, fresh standard solution. The cells were incubated with the indicated agents. Results are expressed as the percent of the radioactivity present at the beginning of incubations. Histamine was at 1 mM and, unless otherwise indicated, all incubations were at 37°. *Inset.* Same results as in B expressed as the natural logarithm of the fraction remaining vs time in min. Each point is the mean of triplicate determinations; this experiment is representative of four others.

cooperative interactions seen in certain other systems [14]. Analysis of the dissociated radioactivity after 40 min of incubation by thin-layer chromatography [15] revealed that over 80 percent migrated as [^{3}H]histamine and was chemically identical to authentic histamine. Reducing the incubation temperature from 37 to 4° decreased the rate of dissociation by 93 percent (0.0038 min^{-1}). The results at 4° indicate that the loss of bound radioactivity during the wash-suspension procedure used to measure binding was negligible.

To examine the possibility that the cell-associated radioactivity might reflect uptake of [^{3}H]histamine into the cell, cells were incubated with [^{3}H]histamine or ^{42}K (used as a cytoplasmic marker) and then hypotonically lysed with 50 vol. of iced distilled water. Following ultrafiltration, we found that lysis of the cells had caused a loss of most of the ^{42}K (94 per cent) but of only a fraction of the [^{3}H]histamine (24 per cent), suggesting that [^{3}H]histamine was not simply taken up but was actually bound to the cells (Table 1). Furthermore, boiling the gastric cell suspension for 5 min abolished [^{3}H]histamine binding as measured by ultrafiltration [5] (results not shown).

Table 1. Effect of hypotonic lysis on cell-associated [^{3}H]histamine and ^{42}K in dispersed gastric mucosal cells*

Agent	Cell-associated radioactivity (% of total)	
	No lysis	Lysis
[^{3}H]Histamine	1.80 ± 0.20	1.40 ± 0.25
Plus 1 mM histamine	0.22 ± 0.02	0.10 ± 0.02
^{42}K	1.30 ± 0.10	0.10 ± 0.01
Plus 1 mM histamine	1.25 ± 0.12	0.09 ± 0.01

* Cells were suspended in standard solution containing [^{3}H]histamine or ^{42}K (New England Nuclear, 64 mCi/mmole) and incubated with or without 1 mM histamine for 30 min at 37°. The cells were then washed with iced standard solution (no lysis) or hypotonically lysed with iced water (lysis). Each value is the mean ± 1 S.D. of three separate experiments.

Since histamine increased cyclic AMP synthesis and [^{14}C]aminopyrine uptake by these cells [3, 5], we examined the relationship between binding of [^{3}H]histamine and histamine stimulation of these two processes. Inhibition of [^{3}H]histamine binding by unlabeled histamine was detected at 2 μM histamine, was half-maximal at 10 μM histamine, and was abolished by 1 mM histamine (Fig. 2).

Plotting the results in Fig. 2 and results from nine other similar experiments in the form described by Scatchard [16] (bound/free vs bound) yielded a single straight line from which it was calculated that gastric cells possessed 62 ± 29 pmole binding sites per 10^6 cells, with half of these sites being occupied at 20 ± 10 μM histamine (mean ± 1 S.D. of ten experiments) (results not shown).

The interaction between [^{3}H]histamine and gastric cells was further investigated by measuring the off-rate and the on-rate of [^{3}H]histamine binding at several [^{3}H]histamine concentrations [17]. The kinetic analysis revealed a K_{on} = 1.8 ± 0.6 × 10^4 $M^{-1} \cdot min^{-1}$ and a K_{off} = 0.08 ± 0.03 min^{-1} (mean ± 1 S.D., N = 4). The kinetically derived affinity constant, $K_a = K_{off}/K_{on}$ = 4.4 × 10^6 M, compared favourably with the apparent affinity values obtained by equilibrium analysis (10 μM) (Fig. 2) and Scatchard analysis (20 ± 10 μM).

Increasing the concentration of histamine in the incubation solution caused a progressive increase in both cellular cyclic AMP and [^{14}C]aminopyrine uptake (Fig. 2). Aminopyrine is a weak base (pK_a 5.0) which accumulates in an acid-containing compartment, and its uptake has been used for qualitative estimation of acid formation by parietal cells *in vitro* [18].

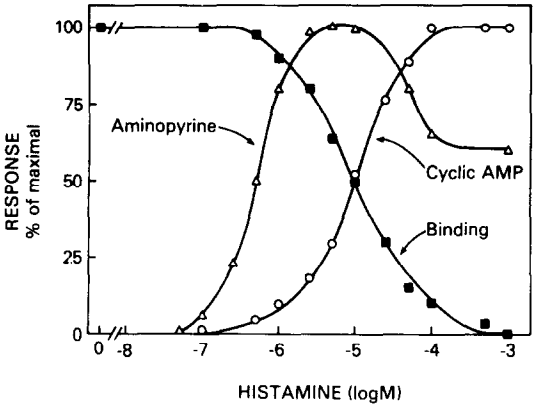

Fig. 2. Effect of histamine on binding of [^{3}H]histamine, cyclic AMP, and [^{14}C]aminopyrine uptake. [^{3}H]Histamine binding and cyclic AMP were determined at 37° after 60 min, and [^{14}C]aminopyrine uptake after 75 min, of incubation. Results for [^{14}C]aminopyrine uptake are expressed as the percent of the increase obtained with 10 μM histamine (1.3 ± 0.2 percent of total, mean ± 1 S.D.) and for cyclic AMP as the percent of the increase obtained with 100 μM histamine (48 ± 7 pmoles/10^6 cells, mean ± 1 S.D.). Binding of [^{3}H]histamine is expressed as percent of the specific binding (i.e. 100 percent) obtained with the tracer alone (0.2 ± 0.02 pmole/10^6 cells). Each point is the mean of triplicate determinations; this experiment is representative of five others.

It appeared from Fig. 2 that there was a good correlation between the ability of histamine to inhibit [^{3}H]histamine binding and to increase cellular cyclic AMP, and for both processes histamine had equal potency (10 μM) (Fig. 2). By comparison, the relation between [^{3}H]histamine binding and [^{14}C]aminopyrine uptake was less apparent. Histamine was approximately 10-fold more potent in stimulating [^{14}C]aminopyrine uptake than in inhibiting [^{3}H]histamine binding, and the dose–response curve for aminopyrine uptake was biphasic. As we discussed elsewhere for cellular cyclic AMP [5], there was a good correlation between these two processes with histamine concentrations of 10 μM or less (correlation coefficient 0.90, $P < 0.01$).

We examined the specificity of [^{3}H]histamine binding to gastric mucosal cells by measuring the ability of various agents to inhibit binding of [^{3}H]histamine. Gastrin, prostaglandin E_1, secretin, glucagon, vasoactive intestinal peptide, and the C-terminal octapeptide of cholecystokinin (all 1 μM), as well as carbachol (1 mM) and atropine (10 μM), did not inhibit binding of [^{3}H]histamine (results not shown). In contrast, three histamine-receptor agonists inhibited [^{3}H]histamine binding. Increasing the concentration of 4-methylhistamine (4MH), a selective H_2-receptor agonist [19], or 2-pyridylethylamine (PEA), a selective H_1-receptor agonist [20], caused a progressive inhibition of [^{3}H]histamine binding (Fig. 3). 4MH was approximately 20-fold more potent than PEA, and the potencies with which they inhibited binding were similar to their potencies in stimulating [^{14}C]aminopyrine uptake [5] or cyclic AMP generation in these cells [4]. That is, a half-maximal effect on [^{3}H]histamine binding occurred with 41 ± 15 μM 4MH and 700 ± 200 μM PEA whereas a half-maximal increase in cellular cyclic AMP occurred at 60 ± 15 μM 4MH and 900 ± 300 μM PEA [4]. A sufficiently high concentration of either agonist could abolish [^{3}H]histamine binding (Fig. 3). Similar results were obtained with 2-methylhistamine instead of PEA. In contrast, tele-methylhistamine—another analogue of histamine—failed to inhibit [^{3}H]histamine binding or to increase cellular cyclic AMP (results not shown). These results suggest that the abilities of these analogues to increase cellular cyclic AMP are the results of their interactions with histamine receptors on gastric cells.

We reported that, in guinea pig mucosal cells, stimulation of cyclic AMP synthesis by histamine was inhibited by H_1- and H_2-antagonists [4]. We examined the ability of cimetidine and promethazine to inhibit [^{3}H]histamine binding and found that increasing the concentration of either antagonist caused a progressive reduction in [^{3}H]histamine binding (Fig. 3). Cimetidine was approximately four times more potent than promethazine and, at high concentrations, both cimetidine and promethazine could abolish [^{3}H]histamine binding. Results similar to those illustrated in Fig. 3 were obtained using metiamide instead of cimetidine and using diphenhydramine, chlorpheniramine or mepyramine instead of promethazine.

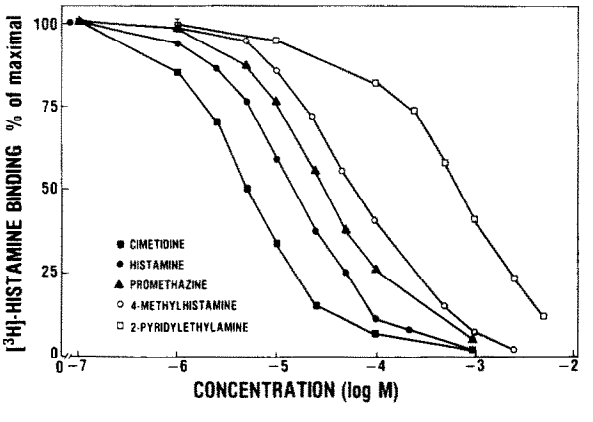

Fig. 3. Effect of histamine-receptor agonists and antagonists on [^{3}H]histamine binding to guinea pig mucosal cells. Cells were incubated with [^{3}H]histamine (35 nM) plus the indicated agents for 60 min at 37°. Results are expressed as percent of the specific binding obtained with the tracer alone (0.22 ± 0.03 pmole/10^6 cells). Each point is the mean of triplicate determinations; this experiment is representative of three others.

The results presented here demonstrate [^{3}H]histamine binding to dispersed mucosal cells isolated from guinea pig stomach. The binding was localized to membrane components that are probably the H_2-receptors on the plasma membrane of parietal cells. Unlike rabbit gastric glands in which uptake of [^{3}H]histamine was reported [21], in our preparation most of the cell-associated radioactivity appeared to result from binding of [^{3}H]histamine.

Both kinetic and thermodynamic analyses of binding indicated the presence of a single class of receptor with no evidence for cooperative interaction between [^{3}H]histamine and the receptors. The lack of cooperativity suggests that our histamine–receptor system represents a simple interaction. This interpretation is supported by the reasonably close agreement between the affinity constants derived from the equilibrium analysis and the kinetic analysis. There was also a good correlation between binding of histamine and histamine stimulation of cyclic AMP synthesis. We concluded that binding of histamine to the receptors is followed by activation of adenylate cyclase and an increase in cellular cyclic AMP.

The potencies of histamine analogues as well as of cimetidine and promethazine on [^{3}H]histamine binding were similar to the potencies with which they affected cellular cyclic AMP [4]. These results indicate that the actions of both agonists and antagonists, to increase cyclic AMP and to inhibit cyclic AMP synthesis, respectively, reflect their interactions with the histamine receptor. The *relative* potencies with which these agents affected [^{3}H]histamine binding and cyclic AMP in guinea pig gastric cells were similar to their *relative* potencies on functions that are considered to be mediated by H_2-receptors (e.g. gastric acid secretion, contraction of atrial or uterine smooth muscle) [19, 20, 22]. Since, in terms of cyclic AMP and [^{3}H]histamine binding in our preparation, these receptors have higher affinity for H_2-agonists or H_2-antagonists than for H_1-agonists or H_1-antagonists, we suggest that [^{3}H]histamine binds to the H_2-receptors that mediate both the rise in cyclic AMP and the acid-secretory response as reflected by [^{14}C]aminopyrine uptake.

[^{3}H]Histamine appears to be a desirable tool for studying the H_2-receptors on isolated parietal cells. Its usefulness in this and other similar preparations can facilitate studies on structure–function relationships of newly synthesized histamine H_2-antagonists which may have important therapeutic applications. Furthermore, [^{3}H]histamine could be useful in solubilization and isolation of the H_2-receptors from parietal cells as has been reported for the H_2-receptors from calf thymocyte membranes [13].

In summary, dispersed mucosal cells from guinea pig stomach were shown to possess a single class of histamine H_2-receptors to which [^{3}H]histamine bound with an affinity constant of 5–25 × 10^6 M^{-1} as determined by either equilibrium or kinetic analysis. Maximal binding capability was 62 ± 29 pmoles/10^6 cells. The binding was reversible and temperature-dependent, and no evidence was found for cooperative interaction between [^{3}H]histamine and the receptor-cell system. This binding of histamine correlated with the ability of histamine to increase cellular cyclic AMP and to stimulate the acid secretory response of parietal cells as reflected by cellular uptake of [^{14}C]aminopyrine.

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Inhibition of benzodiazepine receptor binding by several tryptophan and indole derivatives

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Today, increasing evidence exists that the pharmacological activities of tranquilisers of the benzodiazepine group are mediated via benzodiazepine specific, high-affinity binding sites. These "benzodiazepine receptors" are stereospecific and are only present in the central nervous system, but not in peripheral organs of many vertebrate species, with a pronounced regional distribution [1–4]. However, the physiological function of the benzodiazepine receptor is still unclear mainly because it is still not known which endogenously occurring substance represents the native ligand of this receptor. Inosine, hypoxanthine and nicotinamide have had most attention as possible candidates for the unknown endogeous ligand [5], though their affinities for the benzodiazepine receptor are very low (Table 1). Much higher affinities have been found for some β-carboline derivatives [6–9] whereof norharmane-3-carboxylic acid ethylester is the most potent [6]. However, this compound has not yet been found *in vivo* [6]. At the present state, harmane (1-methyl-β-carboline) is the most potent endogenously occurring [10–12] inhibitor of benzodiazepine receptor binding [7]. Therefore, harmane or another related β-carboline derivative are the most attractive candidates for the unknown endogenous ligand of the benzodiazepine receptor.

While the complete biosynthetic pathway of β-carbolines in animals or man is not known it is quite clear that these