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Histamine-induced inositol phospholipid breakdown in the longitudinal smooth muscle of guinea-pig ileum

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1 The characteristics of histamine-stimulated inositol phospholipid breakdown in slices of guinea-pig ileal smooth muscle and cerebellum have been investigated.

2 In cerebellar slices the inhibition of the inositol phospholipid response to histamine by mepyramine was consistent with competitive antagonism of histamine H_1-receptors.

3 In slices of the longitudinal smooth muscle of guinea-pig ileum, mepyramine produced only a weak inhibition of the response to histamine, at concentrations up to 1 μM. This was in striking contrast to the potent competitive antagonism of the H_1-mediated contractile responses obtained with mepyramine in this tissue.

4 The H_1-receptor antagonists (+)-chlorpheniramine and promethazine similarly had no effect on the EC_{50} value for histamine in guinea-pig ileum, while promethazine competitively antagonized the muscarinic receptor-mediated inositol phospholipid response in this tissue (K_a 3.6×10^7 M^{-1}).

5 Cimetidine, on its own, did not significantly inhibit the inositol phosphate accumulation elicited by histamine in ileum. In the presence of 0.2 μM mepyramine, cimetidine (0.1 mM) produced a small parallel shift of the histamine concentration-response curve (K_a 3×10^4 M^{-1}). This inhibition, however, was not consistent with antagonism of an H_2-receptor-mediated response.

6 The effect of a range of histamine analogues on inositol phospholipid breakdown was determined. Dose-response curves were constructed and characterized in terms of the EC_{50}, slope and maximal response attainable relative to histamine.

7 The H_1-agonists, N^{α},N^{α}-dimethylhistamine, N^{α}-methylhistamine, 2-pyridylethylamine and 2-thiazolylethylamine produced the largest accumulations of [^{3}H]-inositol-1-phosphate. A very weak response was produced by the H_2-selective agonist impromidine, while dimaprit (also H_2-selective) was without significant effect.

8 Mepyramine appeared to antagonize competitively the response to the H_1-selective agonist 2-pyridylethylamine. This was in contrast to the data obtained with other H_1-agonists, where mepyramine produced only a small dextral shift of the agonist curves at low agonist concentrations and an increase in the Hill coefficient. This was particularly striking in the case of 2-methylhistamine.

9 The results suggest that an H_1-receptor component in guinea-pig ileum, may coexist with a larger inositol phospholipid response to histamine which is independent of the activation of H_1- or H_2-receptors.

Introduction

Stimulation of a wide range of cell surface receptors leads to an increase in the intracellular level of calcium ions. An early event which has been reported to be associated with the activation of all calcium mobilizing receptors is the hydrolysis of phosphatidylinositol and the polyphosphoinositides to diacylglycerol and the corresponding inositol phosphates; inositol-1-phosphate, inositol-1-, 4-bisphosphate and inositol-1, 4, 5-trisphosphate (Michell, 1975; 1979; Hawthorne & Pickard, 1979; Berridge, 1981; 1983; Mitchell *et al.*, 1981; Cockcroft, 1981; Putney, 1981). This breakdown of inositol phospholipids has been implicated as a transducing mechanism for controlling the calcium permeability of the plasma membrane (Michell, 1975; 1979; Berridge, 1981; 1983; Michell *et al.*, 1981; Putney, 1981) and, more recently, for the mobilization

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of the intracellular calcium store (Berridge, 1983; Streb *et al.*, 1983; Burgess *et al.*, 1984).

The recent demonstration that inositol phospholipid breakdown can be monitored by following the accumulation of inositol-1-phosphate in the presence of lithium ions (Berridge *et al.*, 1982) has provided a direct and sensitive assay for monitoring agonist-induced inositol phospholipid breakdown in central and peripheral tissues (Berridge *et al.*, 1982; Watson & Downes, 1983; Brown *et al.*, 1984; Bone *et al.*, 1984). Using this technique, histamine H_1-receptor-mediated increases in inositol-1-phosphate accumulation have been demonstrated in lithium-treated slices of rat cerebral cortex (Brown *et al.*, 1984) and guinea-pig cerebral cortex and cerebellum (Daum *et al.*, 1984).

In the longitudinal smooth muscle of guinea-pig small intestine, activation of histamine H_1-receptors leads to contraction by elevating the intracellular concentration of free calcium ions (Bolton, 1979; and references therein). Indirect studies of ^{32}P-labelled phosphate (^{32}Pi) incorporation into phosphatidylinositol have indicated that histamine may also increase phosphatidylinositol breakdown in this tissue and suggested a relationship between the breakdown of inositol phospholipids and histamine H_1-receptor-induced changes in cell surface permeability to calcium ions (Jafferji & Michell, 1976a,b). At present, however, the evidence for an involvement of H_1-receptors in the inositol phospholipid response to histamine in guinea-pig ileal smooth muscle is not strong and previous studies have been limited to the use of a single high concentration (12.5 μM) of mepyramine (Jafferji & Michell, 1976a). In this paper we have examined in greater detail the characteristics of the effect of histamine on inositol phospholipid breakdown in slices of guinea-pig ileal longitudinal smooth muscle, using the more direct measurement of inositol-1-phosphate accumulation. Parallel studies of the H_1-receptor mediated inositol phospholipid response in guinea-pig cerebellum have been made for comparison.

Methods

Accumulation of ^3H-inositol-phosphates

Hartley strain guinea-pigs of either sex (200–400 g) were killed by cervical dislocation and decapitation. Slices (300 × 300 μM) of cerebellum and ileal smooth muscle (from longitudinal muscle strips of guinea-pig small intestine, prepared essentially as described by Rang, 1964) were obtained with a McIlwain tissue chopper. Pooled slices from two or three animals were washed and incubated at 37°C in 30 ml Krebs-Henseleit medium (mM): NaCl 118, KCl 4.7, $MgSO_4$ 1.2, $CaCl_2$ 2.5, KH_2PO_4 1.2, $NaHCO_3$ 25, glucose 5.5, pH 7.4 gassed with O_2/CO_2 (95 : 5). After 30 min the Krebs medium was decanted and the slices resuspended in 5 ml of Krebs medium containing 40 μCi (0.5 μM) [^3H]-myo-inositol and the incubation continued under an atmosphere of O_2/CO_2 (95 : 5) for a further 2 h at 37°C in a shaking water bath. The prelabelled slices were then washed with 20 ml Krebs solution at 37°C every 15 min for a period of 1 h. Ileal slices were finally washed and resuspended in 4 ml of Krebs medium. Aliquots (75 μl) of the ileal slice suspension were added to 165 μl of Krebs medium containing LiCl (final concentration 10 mM) or to Krebs medium containing LiCl and antagonist drug in Beckman Biovials. Cerebellar slices were washed and allowed to settle under gravity. Portions (40 μl) of the gently packed slices were added to 200 μl of Krebs medium containing LiCl and, where appropriate, antagonist drug. The tubes were gassed with O_2/CO_2 (95 : 5), capped and incubated for 30 min at 37°C. Histamine was added after this step in 10 μl of medium, the tubes gassed again with O_2/CO_2 (95:5), and the incubation continued for a further 45 min. The incubations were stopped by addition of 0.94 ml of chloroform/methanol (1 : 2 v/v). Chloroform (0.31 ml) and water (0.31 ml) were then added to separate the phases. A portion (0.75 ml) of the upper aqueous phase was removed, diluted to 3 ml with water and applied to columns containing 0.25 ml Dowex-1 resin in the formate form (applied as 0.5 ml of a 50% v/v slurry in distilled water). The columns were washed with 10 ml of 5 mM myo-inositol to remove [^3H]-inositol and the total ^3H-inositol phosphates were eluted with 1.5 ml 1 M ammonium formate/0.1 M formic acid.

In later experiments glycerophosphoinositol was removed with 5 ml of 5 mM disodium tetraborate/60 mM sodium formate, before the elution of the remaining ^3H-inositol phosphates (almost exclusively [^3H]-inositol-1-phosphate, see Figure 3) with 1.5 ml 1 M ammonium formate/0.1 M formic acid. The 1.5 ml fractions eluted from the columns were counted for radioactivity after addition of 12 ml Biofluor.

For separation and assay of the water soluble phosphorylated inositol derivatives, formed during incubations, the water soluble products were applied to Dowex-1 anion exchange columns (0.25 ml) and eluted with 5 × 1 ml fractions of (1) 5 mM myo-inositol; (2) 5 mM disodium tetraborate/60 mM sodium formate; (3) 0.1 M formic acid/0.2 M ammonium formate; (4) 0.1 M formic acid/0.4 M ammonium formate; (5) 0.1 M formic acid/1.0 M ammonium formate. According to Berridge *et al.* (1983), glycerophosphoinositol, inositol-1-phosphate, inositol-1,4-bisphosphate and inositol-1, 4, 5-trisphosphate are eluted in peaks 2–5 respectively. [^3H]-inositol is not retained and was removed with 5 mM myo-inositol. The tritium

content of 1 ml fractions was determined by scintillation counting in 10 ml Biofluor.

Analysis of data

Concentration-response curves for histamine- or carbachol-induced ^{3}H-inositol phosphate accumulation were fitted to a Hill equation using the program ALLFIT (DeLean *et al.*, 1978). The actual equation fitted was:

$$\text{stimulation of } ^3\text{H-inositol phosphate accumulation} = \frac{E_{max} \times D^n}{D^n + (EC_{50})^n}$$

Where D is the agonist concentration, n is the Hill coefficient, EC_{50} is the concentration of agonist giving half maximal stimulation and E_{max} is the maximal stimulation. Each point was weighted according to the reciprocal of the variance associated with it. ALLFIT was also used to fit families of histamine concentration-response curves, obtained in the presence and absence of antagonist drug, to the same Hill equation. This procedure allowed parameters to be shared between different curves. The programme was implemented on a 64K Apple II europlus using a Pascal version of ALLFIT adapted by Dr Carl Johnson, University of Cincinnati, U.S.A.

Affinity constants for antagonists were obtained from the parallel shift of the log dose-response curves to histamine or carbachol using the relationship:

$$\text{Dose-ratio} = A.K_a + 1$$

where A is the concentration of antagonist, K_a is the affinity constant of the antagonist and the dose-ratio is the ratio of the concentration of agonist necessary to give a specified response in the presence of antagonist to the concentration of agonist required for the same response in the absence of antagonist. Where the data were adequate the dose-ratios obtained were utilized to determine Schild slopes (m) by unweighted linear regression of the Schild equation (Arunlakshana & Schild, 1959):

$$\log(\text{Dose-ratio} - 1) = m\log A + \log K_a$$

Concentration-response curves for certain histamine analogues, obtained in the presence and absence of 0.1 μM mepyramine, were also fitted as double hyperbolae using the Harwell Library non-linear regression program VB01A. The equation fitted was:

$$\text{stimulation of } [^3\text{H}]\text{-inositol-1-phosphate accumulation} = \frac{N_1.D}{K_1 + D} + \frac{N_2.D}{K_2 + D}$$

where D is the agonist concentration, K_1 and K_2 are the respective EC_{50} values of the agonist for the two components and N_1 and N_2 represent the maximum levels of stimulation achieved by each component. For each analogue, the two curves obtained in the presence and absence of 0.1 μM mepyramine were fitted simultaneously with common values of N_1, N_2 and K_2. For the data obtained in the presence of mepyramine, K_1 was set to be a factor of 90 higher than the value of K_1 for the control set of data. This was the dose-ratio expected if mepyramine had an affinity constant of 8.9×10^8 M^{-1} for site 1 (see Discussion). Unweighted non-linear regression analysis was performed simultaneously on the two sets of data using VB01A. For each set of data the non-linear regression routine was directed to the appropriate equation for minimization and associated partial derivatives by the VB01A defined subroutine DERIV in the calling program. Repeated trials were made with different initial parameter estimates and the final best-fit values defined as those that were associated with the lowest residual sum of squares. VB01A was implemented on the Nottingham University ICL 2900.

Organ bath measurements

Longitudinal muscle strips from guinea-pig ileum were suspended in 10 ml of Krebs-Henseleit solution gassed with O_2/CO_2 (95 : 5) at 37°C in a conventional organ bath. Histamine was used as agonist and contractions were recorded isotonically. Histamine was in contact with the tissue for 15–25 s and doses were added at 3 min intervals. Antagonists or lithium chloride were added to the reservoir solution and allowed to equilibrate with the tissue for at least 30 min before subsequent dose-response curves were determined.

Drugs

Myo-[2-^{3}H]-inositol (15.8 Ci mmol^{-1}) was purchased from New England Nuclear. Immediately before use, [^{3}H]-myo-inositol was passed through a column of Dowex-1 resin (formate form) in order to remove radiolytic decomposition products that otherwise interfere with the determination of ^{3}H-inositol phosphates. Histamine dihydrochloride and carbachol chloride were obtained from BDH and mepyramine maleate and Dowex-1-resin (× 8, chloride form 100–200 mesh) from Sigma. Gifts of dimaprit, impromidine, 2-methylhistamine, 4-methylhistamine, N^{α}-methylhistamine, N^{α},N^{α}-dimethylhistamine, 2-pyridylethylamine (2-(2-aminoethyl) pyridine), 2-thiazolylethylamine (2-(2-aminoethyl) thiazole) and cimetidine (all from Smith, Kline and French), promethazine (May and Baker) and (+)-chlorpheniramine (Schering) are gratefully acknowledged. All the histamine analogues were in the form of the dihydrochloride salt, except for impromidine (trihydrochloride).

Results

Lithium and the contractile response to histamine

Lithium (10 mM) had no significant effect on the contractile response of longitudinal smooth muscle strips to histamine. The EC_{50} values of the concentration-response curves for histamine in the presence and absence of lithium were 0.45 ± 0.12 and $0.42 \pm 0.12\,\mu M$ respectively ($n = 6$). Furthermore, the affinity constant of mepyramine, determined from the inhibition of the contractile response to histamine obtained in Krebs-Henseleit medium containing 10 mM LiCl (K_a $8.9 \pm 0.5 \times 10^8\,M^{-1}$; $n = 5$), was very similar to the value obtained in the absence of lithium (K_a $9.5 \pm 1.3 \times 10^8\,M^{-1}$, Schild slope 1.00 ± 0.15; $n = 14$).

Histamine-stimulated accumulation of total ^{3}H-inositol phosphates

In initial experiments the accumulation of the total ^{3}H-inositol phosphate fraction was measured following separation from ^{3}H-inositol by anion-exchange chromatography. Histamine elicited, in 45 min, a dose-related increase in the accumulation of ^{3}H-inositol phosphates in ileal slices (EC_{50} $7.9 \pm 1.6\,\mu M$; $n = 10$) producing a maximal stimulation of 380 ± 30, expressed as a percentage of the ^{3}H-inositol phosphate accumulation in the absence of agonist. This represented a stimulation from a mean basal level of 311 ± 21 d.p.m. to a maximal stimulation of 1104 ± 80 d.p.m. in the presence of 1 mM histamine ($n = 13$). A similar stimulation from 436 ± 20 to 1314 ± 104 d.p.m. ($n = 12$) was obtained with histamine (1 mM) in slices of guinea-pig cerebellum.

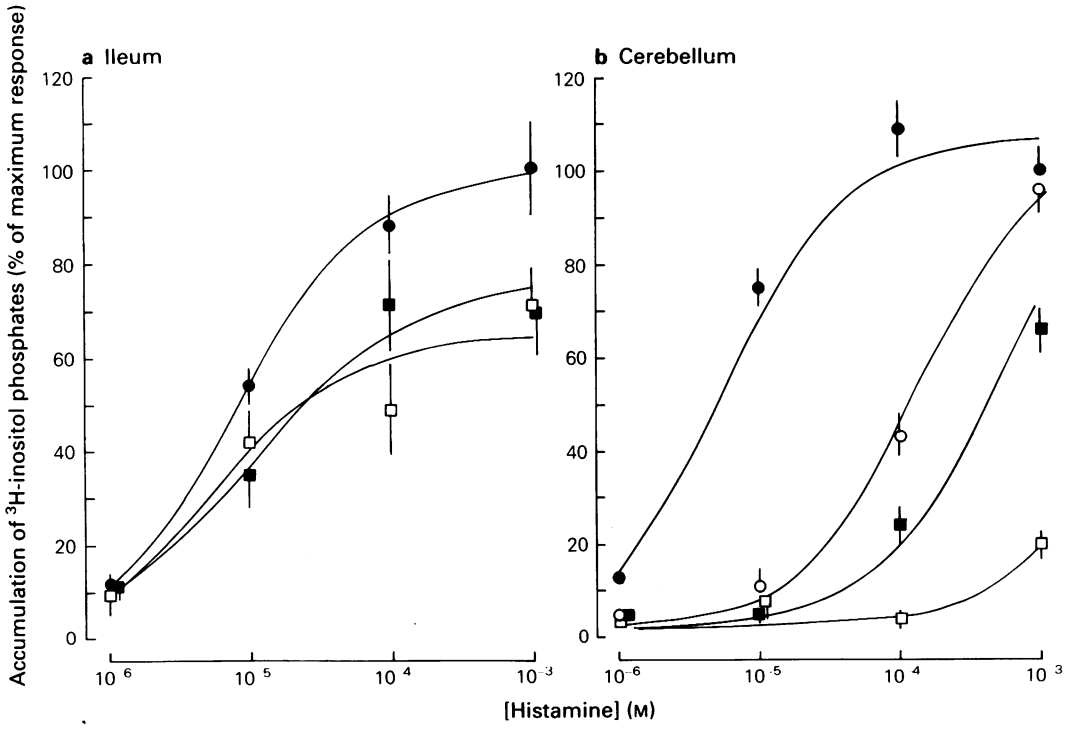

Figure 1 Inhibition by mepyramine of the histamine induced accumulation of total ^{3}H-inositol phosphates in slices of (a) longitudinal smooth muscle of guinea-pig ileum and (b) guinea-pig cerebellum. Concentration-response curves for histamine were obtained in the absence of (●) and presence of (○) 3×10^{-8} M, (■) 10^{-7} M and (□) 10^{-6} M mepyramine. In a given experiment five determinations were made at each of four concentrations of histamine, in the presence and absence of a single concentration of mepyramine. To normalize responses from different slice preparations, responses are expressed as a percentage of the maximal response to histamine (1 mM) obtained in each experiment. Each point represents the combined mean from 2–10 experiments and vertical lines show s.e.means. The curves drawn are the weighted best-fit lines to the logistic equation obtained with ALLFIT, as described under Methods. In (b) the curves drawn are the weighted best-fit lines to the Hill equation with a common slope (0.99 ± 0.12) and maximum response ($106 \pm 6\%$).

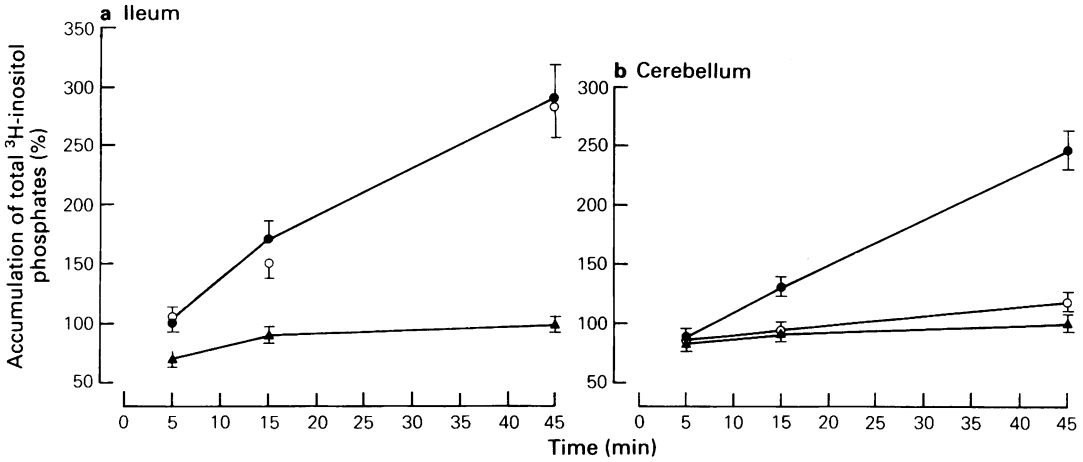

Figure 2 Time course of histamine-induced ^{3}H-inositol phosphate accumulation in (a) guinea-pig longitudinal smooth muscle and (b) guinea-pig cerebellum. (a) (▲) Basal; (●) 1 mM histamine; (○) 1 mM histamine + 0.1 μM mepyramine. (b) (▲) Basal; (●) 0.1 mM histamine; (○) 0.1 mM histamine + 0.1 μM mepyramine. Different concentrations of histamine were used for the two tissues because at the time the experiments were performed, 0.1 mM histamine did not produce a maximal response in ileum (see Figure 7a). To normalize responses from different slice preparations, the accumulation of ^{3}H-inositol phosphates is expressed as a percentage of the basal accumulation following 45 min incubation in each experiment. Each point represents the combined mean of 5 replicates obtained in each of three separate experiments and vertical lines show s.e.means. Experiments were performed as described under Methods, mepyramine being added to the incubations 30 mins before the agonist in every case.

Effect of mepyramine

The selective H_1-receptor antagonist mepyramine produced only a weak inhibition of the histamine-stimulated ^{3}H-inositol phosphate accumulation in guinea-pig ileum at concentrations up to 1 μM (Figure 1a). The inhibition appeared to be non-competitive producing a decrease in the maximal response to histamine (35 ± 9% decrease with 1 μM mepyramine) with no significant effect on the EC_{50} value. Data were also obtained with 3×10^{-8} M mepyramine ($n = 5$), although not included in Figure 1a for the sake of clarity. In the presence of this concentration of mepyramine the best fit value for the maximum response to histamine was 78 ± 7 (expressed as a percentage of the response to histamine alone) and the ratio of the EC_{50} values was 2.8 ± 1.2. In cerebellar slices mepyramine shifted the dose-response curves to histamine to higher agonist concentrations consistent with competitive antagonism (K_a 8.7 ± 0.5 × 10^8 M^{-1}, Schild slope 1.02 ± 0.05; $n = 8$) (Figure 1b).

The accumulation of total ^{3}H-inositol phosphates induced by histamine (0.1 or 1 mM) in both cerebellar and ileal slices increased linearly with time over the period of 45 min used in the present studies (Figure 2). However, in ileum even at the earlier agonist incubation times of 5 and 15 min, there was no significant inhibition of the inositol phospholipid response to histamine by 0.1 μM mepyramine (Figure 2a). This effect was in marked contrast to the large inhibition of the response to histamine observed with mepyramine in cerebellar slices following incubation for 5, 15 or 45 min (Figure 2b).

Accumulation of [^{3}H]-inositol-1-phosphate

To establish whether mepyramine has an inhibitory effect on the accumulation of a minor component of the total ^{3}H-inositol phosphate fraction in ileal slices, the effect of histamine and mepyramine on the accumulation of the individual ^{3}H-inositol phosphates was investigated. Incubation (45 min) of both ileal and cerebellar slices with histamine (0.1 mM) produced a large accumulation of [^{3}H]-inositol-1-phosphate and a smaller increase in the deacylation product [^{3}H]-glycerophosphoinositol (Figure 3). There was no detectable accumulation of [^{3}H]-inositol-1,4 bisphosphate or [^{3}H]-inositol 1,4,5 trisphosphate in either tissue. In cerebellar slices the histamine-induced accumulation of both [^{3}H]-inositol-1-phosphate and [^{3}H]-glycerophosphoinositol was significantly inhibited by 0.1 μM mepyramine (Figure 3b). In contrast, the response to histamine in ileal slices was totally resistant to this concentration of mepyramine (Figure 3a).

In subsequent experiments [^{3}H]-glycerophosphoin-

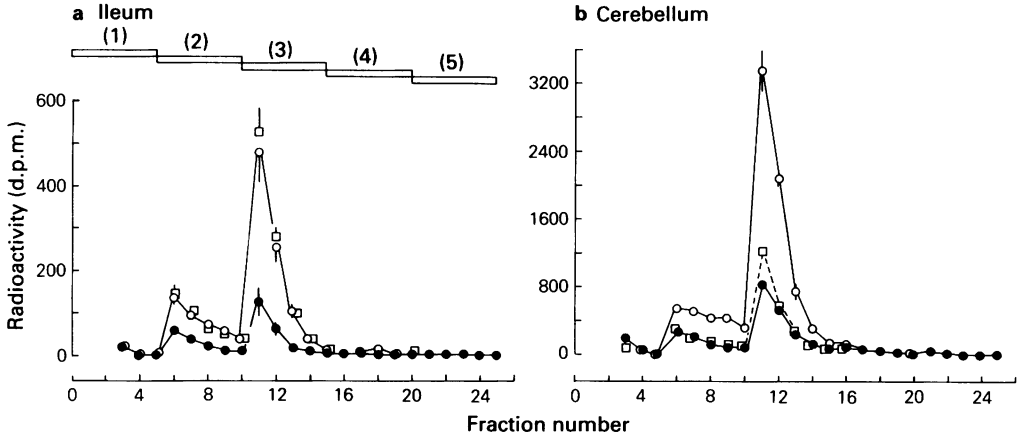

Figure 3 Anion exchange chromatography of ^{3}H-inositol phosphates extracted from slices of (a) longitudinal smooth muscle and (b) cerebellum following stimulation with histamine (45 min) (●) control; (○) 0.1 mM histamine; (□) 0.1 mM histamine + 0.1 μM mepyramine. The water soluble products were applied to Dowex-1 anion-exchange columns and eluted with increasing concentrations of formate as described under Methods. According to Berridge *et al.* (1983), glycerophosphoinositol, inositol-1-phosphate, inositol 1,4 bisphosphate and inositol 1,4,5 trisphosphate are eluted in peaks 2–5 respectively. The bars above (a) (1–5) also apply to the same fraction numbers in (b). Values represent mean of 5 incubations and vertical lines show s.e.mean.

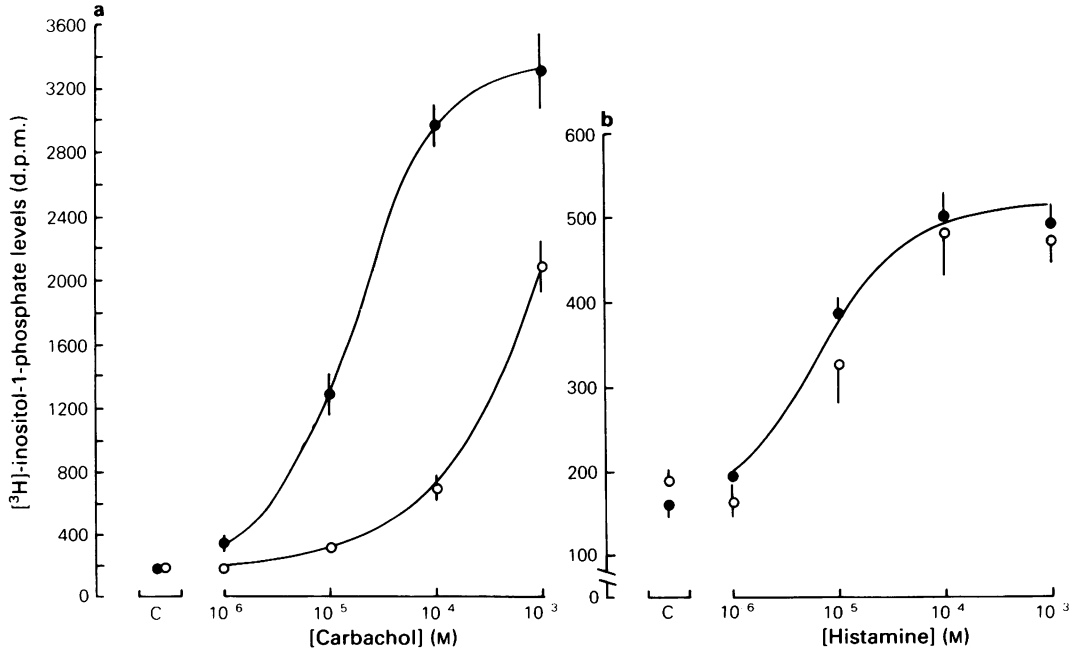

Figure 4 Effect of promethazine on the accumulation of [^{3}H]-inositol-1-phosphate elicited by (a) carbachol and (b) histamine in slices of the longitudinal smooth muscle of guinea-pig ileum. (●) Control; (○) promethazine, 1 μM. Incubations with carbachol or histamine in the presence or absence of promethazine were as described under Methods. The water soluble products were applied to Dowex-1 anion exchange columns and [^{3}H]-inositol and [^{3}H]-glycerophosphoinositol were removed with 5 ml of 5 mM myo-inositol and 5 ml of 5 mM disodium tetraborate/60 mM sodium formate respectively. [^{3}H]-inositol-1-phosphate was eluted with 1.5 ml of 1 M ammonium formate/0.1 M formic acid. Values represent mean of 5 incubations in a single experiment and vertical lines show s.e.mean. Very similar results were obtained in two other experiments.

ositol was removed before the elution of the remaining ^{3}H-inositol phosphate ([^{3}H]-inositol-1-phosphate) from the Dowex anion-exchange columns. The response to histamine in guinea-pig ileum was insensitive to two other H_1-receptor antagonists, (+)-chlorpheniramine and promethazine. The maximal accumulation of [^{3}H]-inositol-1-phosphate elicited by histamine in the presence of 1 μM (+)-chlorpheniramine or promethazine was reduced by 26 ± 5% or 19 ± 6% respectively ($n = 3$ in each case), whilst no significant effect was observed on the EC_{50} values (see for example Figure 4b).

In slices of longitudinal smooth muscle the muscarinic agonist carbachol, elicited a large accumulation of [^{3}H]-inositol-1-phosphate (1600 ± 200%, EC_{50} $1.4 \pm 0.1 \times 10^{-5}$M; $n = 6$) (Figure 4a). This appeared to be a consequence of muscarinic receptor stimulation since the response was sensitive to inhibition by low concentrations of atropine. The affinity constant obtained for atropine, $1.8 \pm 0.2 \times 10^9\,M^{-1}$ ($n = 3$), was in good agreement with the value obtained from antagonism of the contractile response to acetylcholine or carbachol in this tissue, $10^9\,M^{-1}$ (Paton & Rang, 1965; Burgen & Spero, 1968).

To investigate whether the lack of effect of H_1-antagonists on histamine-induced inositol phosphate accumulation was a consequence of their limited diffusion into the ileal slice preparation, we have compared the effect of promethazine on histamine- and carbachol-induced [^{3}H]-inositol-1-phosphate accumulation (Figure 4). Promethazine (1 μM) produced a large parallel displacement of the carbachol dose-response curve to higher agonist concentrations (Figure 4a). The affinity constant deduced for promethazine, for the muscarinic receptor, $3.6 \pm 0.6 \times 10^7\,M^{-1}$ ($n = 3$) was in good agreement with the value ($5 \times 10^7\,M^{-1}$; Bowman & Rand, 1980) obtained in other systems, indicating that promethazine can readily penetrate the slice preparation. However, the greater affinity of promethazine for the histamine H_1-receptor ($1.5 \times 10^9\,M^{-1}$; Bowman & Rand, 1980) was not apparent in studies of histamine-induced [^{3}H]-inositol-1-phosphate accumulation in guinea-pig ileum (Figure 4b).

Effect of cimetidine

The accumulation of [^{3}H]-inositol-1-phosphate elicited by histamine in ileal slices was not markedly inhibited by the H_2-receptor antagonist cimetidine

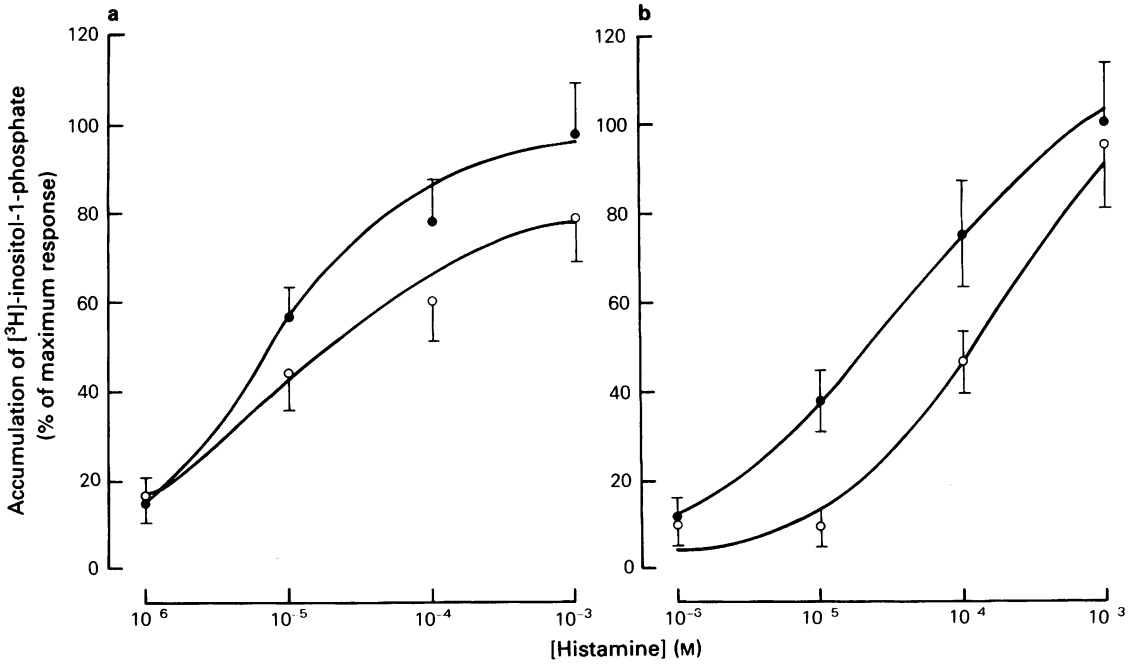

Figure 5 Effect of cimetidine on the accumulation of [^{3}H]-inositol-1-phosphate elicited by histamine in slices of the longitudinal smooth muscle of guinea-pig ileum. (●) Histamine; (○) histamine + cimetidine (0.1 mM). In (b) 0.2 μM mepyramine was present in all incubations 30 min before the addition of histamine as described under Methods. Responses are expressed as a percentage of that produced by 1 mM histamine which was measured in each experiment. Each point represents the combined mean for 5 replicates obtained in each of three separate experiments and vertical lines show s.e.mean.

Table 1 Effect of neurotransmitter antagonists on histamine-induced inositol phospholipid breakdown in guinea-pig ileum

	Accumulation of [^{3}H]-inositol-1-phosphate (d.p.m.)	
	Expt. 1	*Expt. 2*
Basal	171 ± 7	198 ± 17
Histamine	446 ± 60	590 ± 50
Histamine + antagonists		
Phentolamine	576 ± 76	424 ± 57
Atropine	526 ± 86	547 ± 57
Propranolol	500 ± 67	452 ± 112
Naloxone	476 ± 76	533 ± 52
Chlorpromazine	462 ± 98	545 ± 81
Cyproheptidine	438 ± 57	526 ± 93
Theophylline	402 ± 69	550 ± 83

Values represent mean ± s.e.mean of 5 determinations of [^{3}H]-inositol-1-phosphate accumulation. Anatagonists (1 μM) were added to the incubations 30 min before the addition of histamine (1 mM).

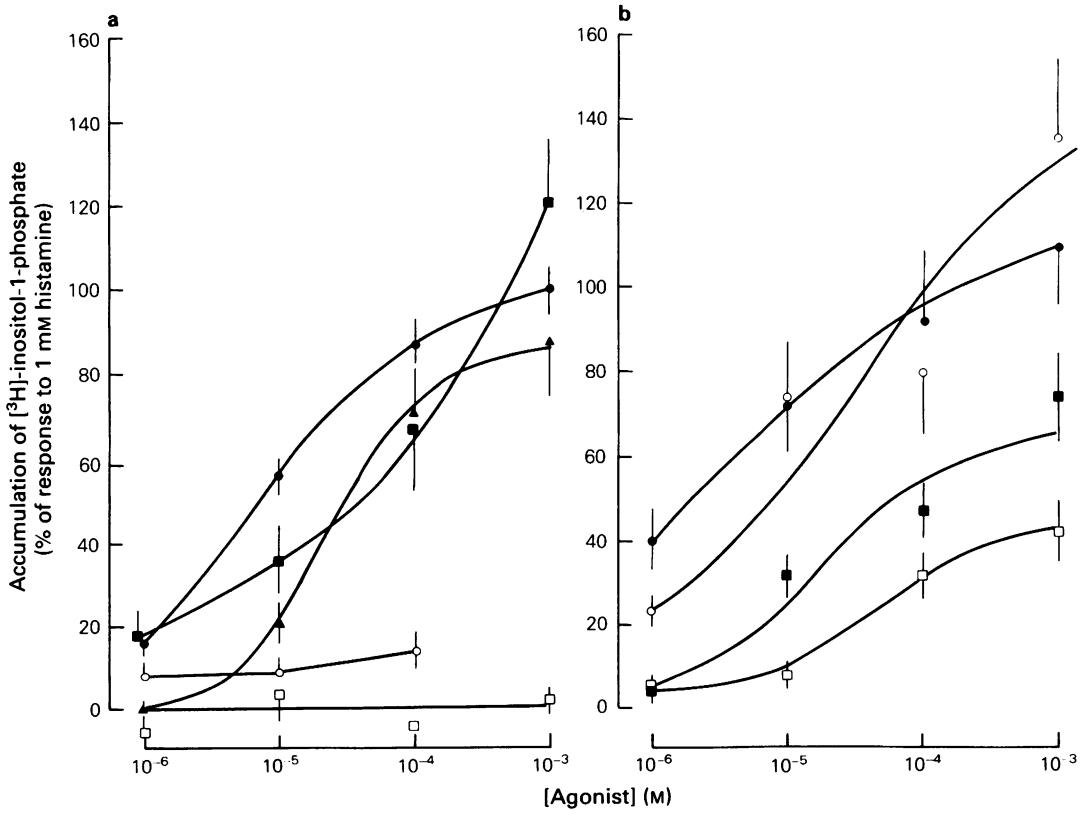

Figure 6 Stimulation of [^{3}H]-inositol-1-phosphate accumulation in slices of ileal smooth muscle by analogues of histamine. To normalize responses from different slice preparations, responses are expressed as a percentage of that produced by 1 mM histamine, which was measured in all experiments. Each point represents the combined mean from 14 (histamine) or 3 (other agonists) separate experiments; vertical lines show s.e.mean. The curves drawn are weighted best-fit lines to the Hill equation (see Methods), except those for dimaprit, impromidine and 2-thiazolylethylamine which were drawn by inspection. (a) (●) Histamine; (▲) 2-pyridylethylamine; (■) 2-thiazolylethylamine; (○) impromidine; (□) dimaprit. (b) (●) N^{α}-Methylhistamine; (○) N^{α},N^{α}-dimethylhistamine; (■) 2-methylhistamine; (□) 4-methylhistamine.

Table 2 Dose-response parameters for the stimulation of inositol phospholipid breakdown in ileal smooth muscle

Agonist	n	EC_{50} (μM)	E_{max} (%)
Histamine	0.81 ± 0.04	7.4 ± 0.8	100 ± 2
N^{α}-Methylhistamine	0.47 ± 0.05	3.8 ± 1.0	120 ± 5
2-Methylhistamine	0.85 ± 0.34	16 ± 15	66 ± 2
2-Pyridylethylamine	1.3 ± 0.3	25 ± 6	86 ± 6
N^{α},N^{α}-Dimethylhistamine	0.44 ± 0.02	53 ± 2	170 ± 97
4-Methylhistamine	0.75 ± 0.28	58 ± 63	49 ± 1

Values (mean ± s.e.mean) of *n* (Hill coefficient), EC_{50} and E_{max}, were obtained from the weighted best fit of the data in Figure 6, to a Hill equation using ALLFIT, as described under Methods. E_{max} is the maximal response relative to the response to 1 mM histamine.

(0.1 mM; Figure 5a), or by a range of other receptor antagonists (Table 1). It is possible that histamine may be stimulating H_1- and H_2-receptors simultaneously and with equal activity. Consequently only minimal inhibition of the response to histamine may be achieved by blockade of one set of receptors. However, following blockade of histamine H_1-receptors with 0.2 μM mepyramine, cimetidine (0.1 mM) produced only a small dextral shift of the dose-response curve to histamine (dose-ratio 4.7; Figure 5b).

Stimulation of [^{3}H]-inositol-1-phosphate accumulation by histamine analogues

Dose-response curves for a range of histamine analogues are shown in Figure 6. It is notable that the maximal response obtained differed markedly among the various compounds tested. In order to make a more quantitative comparison of agonist responses, the concentration-response curves were fitted to a Hill equation and the best fit values obtained for the Hill coefficient (*n*), EC_{50} and the maximal stimulation (Table 2). The data for 2-thiazolylethylamine were inadequate for analysis in this way because the maximal response was insufficiently well defined. A similar problem occurred for N^{α},N^{α}-dimethylhistamine where the uncertainty in the maximal response is reflected in the large error associated with the fitted maximum level (Table 2). Of the compounds tested, 2-thiazolylethylamine, N^{α}-methylhistamine and N^{α},N^{α}-dimethylhistamine produced the largest accumulation of [^{3}H]-inositol-1-phosphate. A very weak response was produced by the H_2-selective agonist impromidine, while dimaprit (also H_2-selective) was without significant effect. Interestingly, the Hill coefficients obtained for histamine, N^{α}-methylhistamine and N^{α},N^{α}-dimethylhistamine were significantly less than the value of unity expected for simple mass action kinetics.

To ascertain whether the response to each of the agonists was sensitive to H_1-antagonists, measurements were made of the inhibition produced by 0.1 μM mepyramine (Figure 7). Mepyramine had little effect on the accumulation of [^{3}H]-inositol-1-phosphate elicited by histamine (Figure 7a). Figure 7b shows the effect of mepyramine on the concentration-response curve to N^{α}-methylhistamine and is representative of the results obtained with N^{α},N^{α}-dimethylhistamine and the H_1-selective 2-thiazolylethylamine. A characteristic feature of these data is the increase in slope of the agonist dose-response curve obtained in the presence of mepyramine associated with a small dextral shift in the position of the curve at low agonist concentrations. This effect is particularly striking in the case of 2-methylhistamine (Figure 7c) where there is a significant inhibition by mepyramine of the [^{3}H]-inositol-1-phosphate accumulation elicited at low agonist concentrations. In contrast, mepyramine appeared to produce a parallel shift of the concentration-response curve for the H_1-selective agonist, 2-pyridylethylamine, to higher agonist concentrations consistent with competitive antagonism (K_a 4.4 ± 1.1 × 10^8 M^{-1}; $n = 3$) (Figure 7d).

Effect of impromidine

In addition to being a selective H_2-receptor agonist, impromidine is a potent antagonist of histamine autoreceptors (putative H_3-receptors) in the rat central nervous system (Arrang *et al.*, 1983). In the present study, however, impromidine (1 μM) did not modify the concentration-response curve to histamine in ileal slices determined in the presence or absence of a combination of 0.2 μM mepyramine and 0.1 mM cimetidine (data not shown).

Discussion

The results presented here show that histamine stimulates the accumulation of [^{3}H]-inositol-1-phosphate in slice preparations from both guinea-pig cerebellum and the longitudinal smooth muscle of

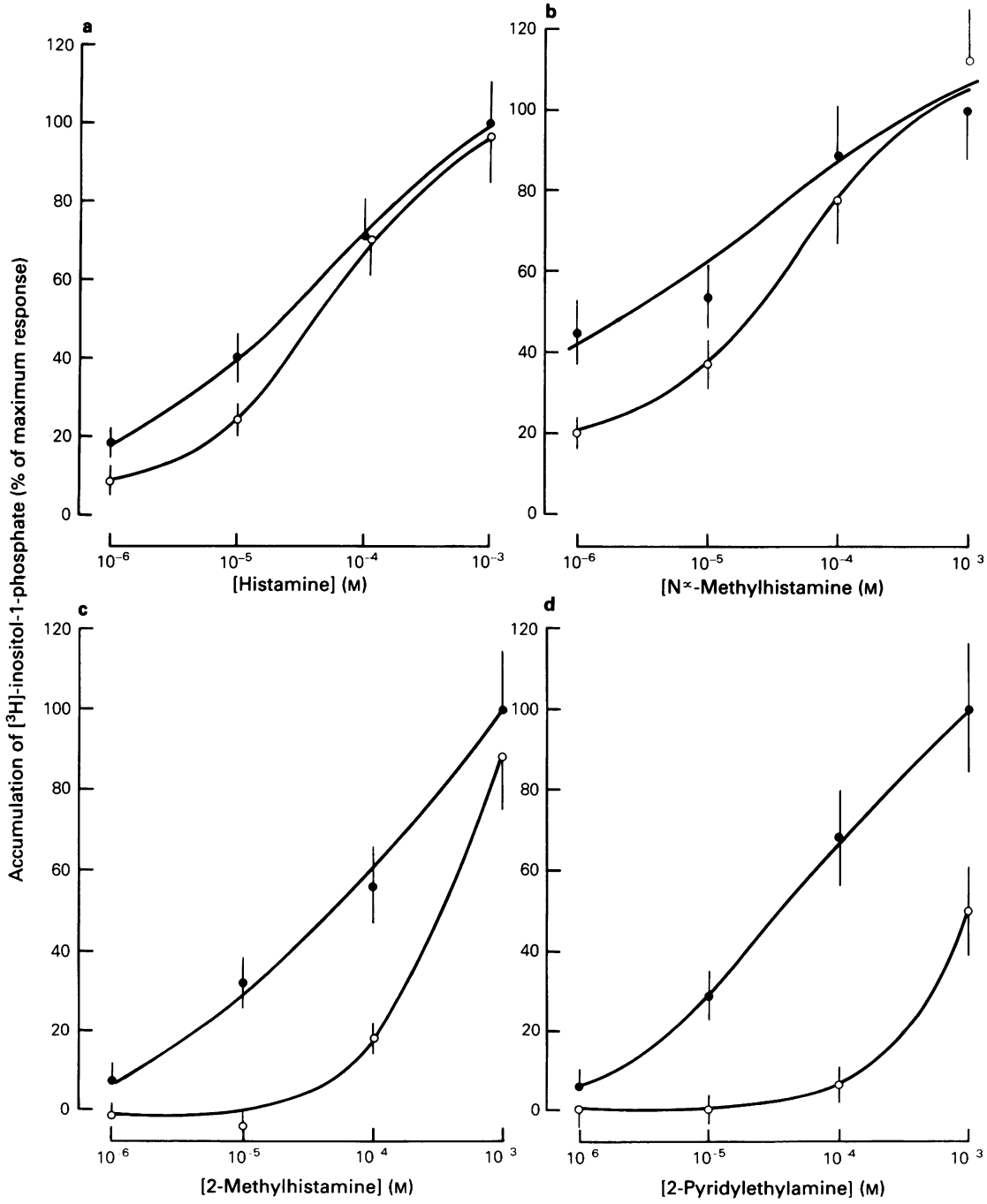

Figure 7 Effect of 0.1 μM mepyramine on the accumulation of [^{3}H]-inositol-1-phosphate elicited by (a) histamine, (b) N^{α}-methylhistamine, (c) 2-methylhistamine and (d) 2-pyridylethylamine. Results are expressed as a percentage of the response obtained with 1 mM agonist in the absence of mepyramine. (●) Agonist; (○) agonist + 0.1 μM mepyramine. Each point represents the combined mean from 3 separate experiments; vertical lines show s.e.mean.

guinea-pig small intestine. In guinea-pig cerebellum there seems little doubt that the accumulation of inositol-1-phosphate induced by histamine is mediated by H_1-receptors. The agreement between the affinity constant obtained for mepyramine from antagonism of histamine-induced inositol phospholidpid breakdown and the value obtained on a classical H_1-receptor system in guinea-pig ileum is particularly striking. This confirms the results obtained in previous studies in guinea-pig brain (Daum *et al.*, 1983; 1984).

In the longitudinal smooth muscle of guinea-pig ileum, the weak and apparently non-competitive nature of the effect of mepyramine on histamine-induced inositol phospholipid breakdown is in striking contrast to the potent competitive antagonism of the H_1-receptor-mediated contractile response obtained with mepyramine in this tissue. It is possible that this lack of effect of mepyramine on the inositol phospholipid response in ileum is due to the presence of lithium ions in the incubation medium. However, the lack of effect of lithium on the characteristics of the contractile response to histamine in this tissue make this explanation unlikely.

An apparent dissociation between histamine-induced inositol phospholipid breakdown and H_1-receptor-mediated contractile activity was observed with two other H_1-receptor antagonists, (+)-chlorpheniramine and promethazine. Both of these agents failed to produce a significant effect on the EC_{50} for histamine at concentrations up to three orders of magnitude higher than those required to produce 50% occupancy of H_1-receptors in this tissue (Hill *et al.*, 1977; Hill & Young, 1981). Studies of the muscarinic antagonist properties of the latter compound provided an opportunity to elucidate whether limited diffusion of these compounds into the ileal slice preparation or other features of the experimental design contributed to the resistance of the response to H_1-receptor antagonists. In ileal slices the muscarinic agonist carbachol elicited a large accumulation of [^{3}H]-inositol-1-phosphate which was consistent with a muscarinic receptor response. The affinity constants deduced for promethazine from inhibition of carbachol-induced phospholipid breakdown and contraction were in good agreement ($3.6 \times 10^7\,M^{-1}$ and $5 \times 10^7\,M^{-1}$ for inositol phospholipid breakdown and contraction respectively). These results suggest that the concentration of promethazine reaching the muscarinic receptors in the lithium treated ideal slice preparation was not significantly lower than that achieved during measurement of contractile activity. Therefore it seems unlikely that differneces in the diffusional characteristics of the two tissue preparations could explain the marked differences in antagonist potency observed with H_1-antagonists. Furthermore, the fact that identical studies if the histamine-induced inositol phospholipid response in guinea-pig cerebellum revealed only an H_1-receptor response suggests that the resistance of the ileal response to mepyramine is not attributable to the experimental protocol.

In addition to H_1-receptors on smooth muscle cells, there are also H_2-receptors on myenteric interneurones which can mediate contractile activity by the release of other contractile agents including 5-hydroxytryptamine and substance P (Barker & Jones-Ebersole, 1982; Barker & Hough, 1983). Although the accumulation of [^{3}H]-inositol-1-phosphate elicited by histamine in this tissue was not markedly inhibited by cimetidine (0.1 mM), it is possible that histamine may stimulate inositol phospholipid breakdown via the simultaneous activation of H_1- and H_2-receptors. If the agonist potency of these two receptors were similar and the overall contribution of the two components equal then only minimal inhibition of the response may be achieved by blockade of one set of receptors with mepyramine or cimetidine. However, in the presence of 0.2 μM mepyramine, cimetidine (0.1 mM) produced a small dextral shift of the dose-response curve for histamine. The calculated affinity constant obtained for cimetidine ($3.7 \times 10^4\,M^{-1}$), assuming competitive antagonism, was very different from the value of $1.3 \times 10^6\,M^{-1}$ expected from antagonism of histamine H_2-receptors (Brimblecombe *et al.*, 1975). This suggests that the inhibitory effect of cimetidine on this response is not a consequence of H_1- or H_2-receptor blockade. Furthermore the H_2-selective agonist impromidine produced a maximal response of only $14.3 \pm 4.2\%$ ($n = 3$) of that achieved with 1 mM histamine, while dimaprit, another selective H_2-agonist, was without significant effect. These results suggest that it is unlikely that the size of the H_2-component, if it is present at all in the inositol phospholipid response to histamine, is sufficient to mask the effect of H_1-receptor blockade. An involvement of putative H_3-receptors also seems unlikely since impromidine (1 μM), which is also a potent inhibitor of H_3-receptors in rat brain (Arrang *et al.*, 1983), was unable to modify the response to histamine in the presence or absence of H_1- and H_2-receptor blockade.

A possibility which also deserves consideration, however, is that an H_1-receptor component may coexist with a larger inositol phospholipid response to histamine which is independent of the activation of the currently established classes of histamine receptor. This latter hypothesis is supported by the results obtained with analogues of histamine. In particular, the data obtained with 2-pyridylethylamine indicate that a major portion of the response to this particular agonist is mediated by H_1-receptors. The affinity constant deduced for mepyramine from inhibition of the inositol phospholipid response to 2-pyridylethylamine, $4.4 \times 10^8\,M^{-1}$, was in reasonable agreement with the value of $8.9 \times 10^8\,M^{-1}$ obtained from inhibi-

tion of H_1-receptor-mediated contractile activity in lithium-treated segments of guinea-pig ileal smooth muscle. What little difference there is between the two values may be accounted for by the presence of a mepyramine resistant component, similar to that observed with other analogues, at high agonist concentrations. If it is accepted that 2-pyridylethylamine is mediating much of its effect through activation of histamine H_1-receptors then this clearly has implications for the responses obtained with other analogues of histamine which are known to have significant H_1-receptor activity. Of the compounds tested N^{α}-methylhistamine, N^{α},N^{α}-dimethylhistamine, 2-thiazolylethylamine and 2-methylhistamine have marked H_1-receptor agonist activity in guinea-pig ileum (Durant *et al.*, 1975; Daum *et al.*, 1982). The relative potencies of these agents for the ileal H_1-receptor being 134, 83, 22 and 20 respectively (all expressed with respect to histamine = 100; Daum *et al.*, 1982). These compounds are more potent than 2-pyridylethylamine (relative potency, 10) so that it would be expected that all of these agents will stimulate H_1-receptors in guinea-pig ileum over the concentration range employed here. It is striking that, with the exception of 2-pyridylethylamine, the concentration-response curves for many of the agonists tested have Hill coefficients less than unity. For the more potent agents; N^{α}-methylhistamine, N^{α},N^{α}-dimethylhistamine and histamine itself, this deviation from simple mass action kinetics was significant ($P < 0.05$). Furthermore, the small dextral shift in the position of the agonist dose-response curves obtained in the presence of 0.1 μM mepyramine was normally accompanied by an increase in Hill coefficient. This was particularly striking in the case of 2-methylhistamine where there was a significant inhibition by mepyramine of the inositol phospholipid response at low agonist concentrations. These results could be explained by the presence of two components in the response involving activation of both H_1-receptors and a second component which is independent of H_1- and H_2-receptor activation. In earlier experiments, the effect of H_1-receptor antagonists on the histamine-induced accumulation of inositol phosphates was characterized by a 18 to 35% reduction in the maximal response rather than the effects observed above. However, these results are not inconsistent with such a two component model, although they suggest that the relative positions of the EC_{50} values for each component may have varied over the 12 months of this study.

To gain an indication of the likely contributions of the different components in the final response, the data in Figure 7 have been fitted to a two site model as described under Methods. For each agonist, the curves obtained in the presence and absence of mepyramine, were fitted simultaneously with common values for the proportions of the two sites (N_1 and N_2) and the EC_{50} value of the non-H_1-receptor component (K_2). The ratio of the EC_{50} values (K_1) for the H_1-receptor component obtained in the presence and absence of antagonist was set at 90, based on an affinity constant of $8.9 \times 10^8\ M^{-1}$. The values for the fitted parameters to the curves obtained with histamine, 2-methylhistamine and N^{α}-methylhistamine are set out in Table 3. A feature of this analysis is the apparent difference in the relative magnitude of the H_1-component between different agonists. In the case of 2-pyridylethylamine this analysis failed to detect any other component, while at the other extreme the magnitude of the H_1-component in the response to histamine was only 23%. The mechanism underlying this difference is not clear. Whether this involves differences in desensitization, penetration into the tissue as a result of uptake into cells (Zilletti *et al.*, 1978) and metabolism, or is a consequence of an interaction between the two components at the level of the effector system, remains to be established.

The exact role of the agonist-induced inositol

Table 3 Parameters of agonist dose-response curves obtained in the presence and absence of mepyramine, fitted to a two site model

Agonist	N_1 (%)	K_1 (M)	N_2 (%)	K_2 (M)
2-Methylhistamine	31 ± 1	$4.7 \pm 0.6 \times 10^{-6}$	72 ± 12	$1.0 \pm 0.4 \times 10^{-3}$
Histamine	23 ± 5	$9.4 \pm 7.9 \times 10^{-8}$	79 ± 6	$5.4 \pm 1.5 \times 10^{-5}$
N^{α}-Methylhistamine	53 ± 7	$2.4 \pm 1.6 \times 10^{-8}$	76 ± 8	$6.3 \pm 2.9 \times 10^{-5}$

Values (mean ± s.e.mean) of the EC_{50}s of the agonist for the 2 components (K_1 and K_2) and of the maximal levels of stimulation achieved by each component relative to the response to 1 mM histamine (N_1 and N_2) (histamine = 100), were obtained from the unweighted best fit to a 2 site model, as described under Methods, of the data in Figure 7. The data obtained for 2-thiazolylethylamine and N^{α},N^{α}-dimethylhistamine were inadequate for analysis in this way, as the maximal response was insufficiently well defined. For each agonist, the two curves obtained in the presence and absence of 0.1 μM mepyramine were fitted simultaneously with common values of N_1, N_2 and K_2.
For the data obtained in the presence of mepyramine, K_1 was set to be a factor of 90 higher than the value of K_1 for the control set of data. Thus for the agonist curves obtained in the presence of mepyramine the values of N_1, N_2 and K_2 are as above, whilst K_1 values are a factor of 90 greater than those shown.

phospholipid breakdown in this tissue and, in particular, whether it is part of a calcium gating mechanism (Michell, 1975; 1979) remains uncertain. The marked difference in the size of the inositol phospholipid responses to carbachol and histamine is consistent with the difference in magnitude of their effects on ^{42}K-efflux from this tissue (Bolton & Clark, 1981; Bolton *et al.*, 1981) and of the differences in H_1- and muscarinic receptor number as deduced from binding studies (Hill *et al.*, 1977; Hill & Young, 1981). It is possible that the difference in magnitude of the inositol phospholipid response to histamine and carbachol is associated with the opening of receptor-operated calcium channels, since these agonists appear to differ in the extent to which they depolarize inner cells by electrotonic spread (Bolton & Clark, 1981). Alternatively, the size of the inositol phospholipid response may reflect a differing ability to release bound calcium from intracellular stores (Takayanagi *et al.*, 1977; Casteels & Raeymaekers, 1979). However, the presence of a significant non-H_1-component in the inositol phospholipid response to histamine in ileal smooth muscle suggests that other roles, perhaps involving the release of arachadonic acid for prostaglandin and leukotriene synthesis (Berridge, 1981; 1983; Rubin *et al* 1981) or the production of diacylglycerol (Berridge 1981; 1984; Nishizuha, 1983; 1984), must be considered.

In summary, the observations presented above suggest that there is an H_1-receptor population in this tissue which mediates contraction in response to histamine and which probably elicits an inositol-phospholipid response. However, this inositol phospholipid response is largely obscured by another, non-H_1-receptor, response to histamine which may be mediated via a hitherto unknown class of receptors. The development of compounds able to selectively stimulate or inhibit the H_1-independent component of the response to histamine in guinea-pig ileum will provide valuable tools for elucidating the precise function of inositol phospholipid breakdown in this tissue.

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