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Regional variation in the characteristics of histamine H_1-agonist mediated breakdown of inositol phospholipids in guinea-pig brain

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1 The position of dose-response curves for histamine-induced accumulation of [^{3}H]-inositol 1-phosphate ([^{3}H]-IP_1) in lithium-treated slices of guinea-pig brain prelabelled with [^{3}H]-inositol differed significantly between cerebellum (EC_{50} 5.1 ± 1.0 μM) and cerebral cortex (EC_{50} 16.3 ± 0.7 μM). The Hill coefficients of the curves, 1.33 ± 0.28 and 1.24 ± 0.03, respectively, did not differ significantly.

2 2-Methylhistamine, N^{α},N^{α}-dimethylhistamine and betahistine were partial agonists in both cerebellum and cerebral cortex, but all produced a greater percentage of the maximum response to histamine in cerebellar slices.

3 In hippocampal slices the response of the partial agonists was intermediate between that in cerebellum and that in cerebral cortex.

4 The four agonists produced an appreciable accumulation of [^{3}H]-inositol 1-phosphate in cerebellar slices even in the absence of Li^+ ion. The EC_{50} and Hill coefficients characterizing the dose-response curves for the four agonists were the same whether 10 mM LiCl was present or not.

5 The affinity constant for mepyramine inhibition of the histamine-induced response was similar in cerebellum, $4.2 \pm 0.6 \times 10^8\ M^{-1}$, and cerebral cortex, $4.6 \pm 1.0 \times 10^8\ M^{-1}$. Curves of mepyramine inhibition of the responses to a fixed concentration of histamine gave no indication of any second component in the response to histamine in either cerebellum or cerebral cortex.

6 The parameters of histamine inhibition of [^{3}H]-mepyramine binding were similar in homogenates of guinea-pig cerebellum and cerebral cortex.

7 These results indicate that H_1-agonist-induced accumulation of IP_1 may not be as directly related to agonist-receptor interaction as simple reaction schemes suggest.

Introduction

Histamine H_1-agonist-induced breakdown of inositol phospholipids may play an essential role in H_1-receptor function, in common with the action of agonists on other 'calcium mobilizing' receptors (Michell, 1975; Berridge, 1984; Berridge & Irvine, 1984; Nishizuka, 1984). Measurement of the agonist-induced breakdown thus offers a particularly promising approach in studies of H_1-receptor function. This is especially true in CNS tissues, where other biochemical responses to H_1-agonists are limited in number and apparently restricted to certain brain regions in certain species (reviewed by Schwartz *et al.*, 1980; 1982).

The breakdown of inositol phospholipids induced by histamine, measured as an increase in the level of [^{3}H]-inositol 1-phosphate ([^{3}H]-IP_1) in lithium-treated slices of regions of guinea-pig or rat brain prelabelled with [^{3}H]-inositol (Berridge *et al.*, 1982), is largely or wholly mediated by H_1-receptors (Daum *et al.*, 1983; 1984; Brown *et al.*, 1984; Donaldson & Hill, 1985; Claro *et al.*, 1986), in accord with the inhibitory effect of single large doses of H_1-antagonists described in earlier studies on the enhanced incorporation of ^{32}Pi or ^{33}Pi into inositol phospholipids in rat brain following the intracerebroventricular injection of histamine (Friedel & Schandberg, 1975; Subramanian *et al.*, 1980). Further, the extent of the histamine-induced accumulation of [^{3}H]-IP_1 in different regions of guinea-pig brain correlates well with the relative density of H_1-receptors determined from the binding of [^{3}H]-mepyramine (Daum *et al.*, 1983; Carswell *et al.*, 1985). This lends some support to the proposition that the reaction is invariably associated with H_1-receptor

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activation (Michell, 1975). However, if histamine-induced accumulation of [^{3}H]-IP_1, measured over an extended incubation period, is to be of maximum utility in studies of H_1-receptor function, and in particular in relating agonist binding to agonist action, then the hydrolysis step should be closely coupled to receptor activation and [^{3}H]-IP_1 formation must reflect this receptor-coupled step. There is some evidence that this may be true in guinea-pig cerebral cortex, since the EC_{50} for histamine-induced accumulation of [^{3}H]-IP_1 is close to the apparent K_D for histamine inhibition of [^{3}H]-mepyramine binding (Daum *et al.*, 1984). However, if the assumptions underlying the use of the simple assay are correct, then this should be generally true and the parameters characterizing the H_1-agonist-induced response should be the same in all brain regions. To test this we have examined the response to histamine and three other H_1-agonists in lithium-treated slices from three regions of guinea-pig brain. Some of the results of this study have been presented in preliminary form (Carswell & Young, 1985).

Methods

Measurement of the accumulation of [^{3}H]-inositol phosphates

Accumulation of [^{3}H]-inositol phosphates in slices from guinea-pig (Dunkin-Hartley strain, males, 300–400 g) cerebellum, cerebral cortex and hippocampus was measured essentially as described by Berridge *et al.* (1982). Cross-chopped slices (350 × 350 μm), prepared on a McIlwain tissue chopper, were washed 3 times and then incubated at 37°C for 60 min in Krebs-Henseleit medium (in mM: NaCl 116, KCl 4.7, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, $CaCl_2$ 2.5 and D-glucose 11) with 3 further changes of medium. The medium was bubbled throughout with 95% O_2 plus 5% CO_2. The slices were washed once more and then transferred to a flat-bottomed vial (Hughes & Hughes Ltd, scintillation vial insert) and allowed to settle under gravity. Portions of the slices (40 μl) were added to 200 μl Krebs-Henseleit medium containing 0.33 μM *myo*-[2-^{3}H]-inositol (1 μCi per incubation) and 10 mM LiCl, in insert vials. The mixture was incubated for 30 min in a shaking water bath before addition of 10 μl of agonist solution or 10 μl Krebs-Henseleit medium. After incubation for a further period, usually 60 min, the reaction was terminated by addition of 0.94 ml chloroform/methanol (1:2, vol/vol) and mixed thoroughly. Chloroform (0.31 ml) and 0.31 ml water were added and the phases separated by centrifugation at 950 *g* for 5 min. A portion of the upper phase (0.8 ml) was applied to a column containing 2 ml of an approximately 1:1 slurry of Dowex-1 anion-exchange resin (formate form) and distilled water. The column was washed with 5 ml water to remove [^{3}H]-inositol, followed by 8 ml 60 mM ammonium formate/5 mM sodium tetraborate to remove any [^{3}H]-glycerophosphoinositol. [^{3}H]-IP_1 was then eluted with 8 ml 200 mM ammonium formate/100 mM formic acid. In some experiments inositol bisphosphate ([^{3}H]-IP_2) and trisphosphate ([^{3}H]-IP_3) were eluted with 8 ml 400 mM ammonium formate/100 mM formic acid and 8 ml 1 M ammonium formate/100 mM formic acid, respectively. In two experiments [^{3}H]-IP_3 was eluted with 8 ml 800 mM ammonium formate/100 mM formic acid and 8 ml 1 M ammonium formate then added to elute any [^{3}H]-IP_4. Aquasol-2 (8 ml) was added to each fraction and tritium determined by liquid scintillation counting.

In some experiments incubations were terminated by addition of 200 μl ice-cold 15% trichloroacetic acid and allowed to stand on ice for 15 min. The mixture was centrifuged (950 *g* for 5 min) and a 250 μl sample of the supernatant taken for analysis. Trichloroacetic acid was removed by extracting 5 times with ether and the aqueous layer neutralized with Na tetraborate before separation of the [^{3}H]-inositol phosphates as above.

Histamine inhibition of [^{3}H]-mepyramine binding

Preparation of membrane fractions from guinea-pig cerebellum and cerebral cortex and measurement of histamine inhibition of [^{3}H]-mepyramine binding was carried out essentially as described previously (Aceves *et al.*, 1985), except that incubations were for 20 min at 37°C. The concentration of [^{3}H]-mepyramine was 0.37–0.57 nM and non-specific binding was determined with 2 μM promethazine. Pentuplicate measurements were made at 12–14 histamine concentrations.

Analysis of data

Concentration-response data for histamine H_1-agonist-induced [^{3}H]-IP_1 accumulation (after subtraction of the level in the absence of agonist) were fitted to a Hill equation (logistic equation) using the Harwell Library non-linear regression programme VB01A. The actual equation fitted was: [^{3}H]-IP_1 accumulated = $Resp_{max}$, $D^n/(D^n + EC_{50}^n)$, where D is the agonist concentration, n is the Hill coefficient, EC_{50} is the dose giving the half-maximal response and $Resp_{max}$ is the maximum response. Each point was weighted according to the reciprocal of the variance associated with it. 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.

The response to a set concentration of histamine

(200 μM with cerebellar and cerebral cortical slices and 500 μM with hippocampal slices) was measured in all experiments with the other H_1-agonists and used to relate the response induced by the agonist to the maximum response to histamine. The occupancy of the set concentration of histamine in each region was calculated assuming a hyperbolic relationship, with the apparent dissociation constant equal to the best-fit value of the EC_{50} in that region.

The affinity constant of mepyramine for the H_1-receptor was determined from the parallel shift of the dose-response curve to histamine, using the relationship: dose-ratio = [A].Ka + 1, where [A] is the concentration of mepyramine and the dose-ratio is the ratio of the dose of agonist required to produce a given response in the presence of antagonist to that required in its absence.

Curves of the inhibition of the response to a fixed concentration of histamine by increasing concentrations of mepyramine and curves of histamine inhibition of [^{3}H]-mepyramine binding were fitted as described previously (Aceves *et al.*, 1985) using weighted non-linear regression analysis with the Hill coefficient, the IC_{50} (the concentration of mepyramine or histamine required for 50% inhibition of the mepyramine-sensitive or histamine-sensitive component of the response) and the percentage of the response insensitive to inhibition by mepyramine or promethazine as unknowns.

Drugs

Myo-[2-^{3}H]-inositol (16.3 Ci mmol^{-1}) and Aquasol-2 were purchased from New England Nuclear and [pyridinyl-5-^{3}H]-mepyramine (26 Ci mmol^{-1}) from Amersham International. Histamine dihydrochloride and tetrodotoxin were obtained from Sigma and mepyramine maleate from May and Baker. 2-Methylhistamine and N^{α},N^{α}-dimethylhistamine were kindly provided by Smith, Kline and French and betahistine by Duphar laboratories. 5, 8, 11, 14-Eicosatetraynoic acid (ETYA) was kindly provided by Prof S. Nahorski.

Results

Histamine-induced [^{3}H]-inositol 1-phosphate accumulation in brain regions

Histamine induced an accumulation of [^{3}H]-IP_1 in the three brain regions examined. The extent of the stimulation, expressed as accumulation in the presence of histamine minus the basal accumulation, varied quite markedly between slice preparations, although the variation was less if the stimulation was expressed as a percentage of the basal level. The mean values of the extent of the histamine-induced and basal accumulations over a number of experiments are set out in Table 1. The efficiency of the extraction of [^{3}H]-IP_1, measured on cerebral cortical slices, was essentially the same whether the chloroform/methanol or trichloroacetic acid/ether methods were used.

The time course of the accumulation of [^{3}H]-IP_1 induced by 200 μM histamine, measured between 15 and 120 min, was qualitatively similar in cerebral cortical and cerebellar slices and corresponded well with previous measurements in cerebral cortex (Daum *et al.*, 1984). However, the position of the dose-response curve to histamine differed significantly between cerebellar and cerebral cortical slices (Figure 1). The best-fit values for the EC_{50} were 5.1 ± 1.0 μM in cerebellum and 16.3 ± 0.7 μM in cerebral cortex. The Hill coefficients of the two curves, 1.33 ± 0.28 in cerebellum and 1.24 ± 0.03 in cerebral cortex, did not differ significantly. The absence of any obvious secondary response to histamine at high concentrations in cerebellar slices was checked in 2 experiments in which measurements were made between 50 μM and 1 mM histamine.

Regional differences in the response to other H_1-agonists

The difference in the response to histamine between cerebellum and cerebral cortex was reflected by differences in the accumulation of [^{3}H]-IP_1 induced by 2-methylhistamine, N^{α},N^{α}-dimethylhistamine and beta-

Table 1 Histamine-induced accumulation of [^{3}H]-inositol 1-phosphate ([^{3}H]-IP_1) in regions of guinea-pig brain

	[^{3}H]-IP_1 accumulated (d.p.m.)		
Region	*Basal*	*Histamine-induced*	
Cerebellum	4013 ± 389	18071 ± 1448	(21)
Cerebral cortex	1032 ± 117	3116 ± 312	(34)
Hippocampus	1169 ± 180	4018 ± 668	(17)

Values are the basal level of [^{3}H]-IP_1 ± s.e.mean (no histamine present) and the maximum accumulation ± s.e.mean induced by histamine after subtraction of the basal level, with the number of measurements in parentheses. The values for the maximal response were derived from measurements with 200 μM histamine (cerebellum and cerebral cortex) or 500 μM histamine (hippocampus) and the maximum calculated by dividing by the fractional receptor occupancy, determined assuming a dissociation constant for histamine of 5 μM in cerebellum, 16 μM in cerebral cortex and 6 μM in hippocampus (see text). Incubations in the presence of 10 mM Li^+ with or without histamine were for 60 min.

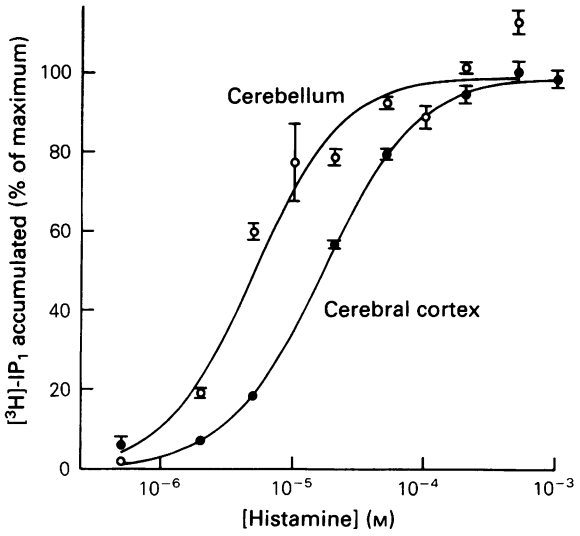

Figure 1 Histamine-induced accumulation of [^{3}H]-inositol 1-phosphate ([^{3}H]-IP$_1$) in cerebellar and cerebral cortical slices. Each point is the weighted mean from 2–9 determinations in cerebellum and 3–20 determinations in cerebral cortex. Vertical lines represent ± approximate s.e.mean. Where no vertical lines are shown the error was within the size of the symbol. The response to 200 μM histamine was measured in all experiments and responses to other concentrations of histamine were expressed as a percentage of this value. The combined values were scaled so that the best-fit maximum of each combined curve was 100%. Details of the equation fitted are given under Methods. Tissue slices were exposed to histamine for 60 min in the presence of 10 mM LiCl. (○) Cerebellar slices; (●) cerebral cortical slices.

histine. All three were partial agonists in both tissues, but achieved a significantly greater maximal effect in cerebellar slices (Figure 2). The difference was particularly apparent with betahistine, which achieved only 8 ± 1% of the maximum response to histamine (= 100%) in cerebral cortical slices, but 48 ± 6% in cerebellar slices.

In the presence of 1 mM betahistine the dose-response curve for histamine in cerebral cortical slices was shifted to the right (Figure 3) in a manner consistent with the action of betahistine as a partial agonist at the H_1-receptor. If the weak agonist action is neglected, the affinity of betahistine deduced from the shift is 7×10^3 M^{-1}, but this value will be an underestimate.

The action of the four H_1-agonists was also examined on slices of hippocampus. The best-fit value for the EC_{50} for histamine from the combined data from 6 experiments was 5.5 ± 0.4 μM (Hill coefficient 1.62 ± 0.18). However, in 2 other experiments, not included in this set, the dose-response curves were shifted markedly to the right (EC_{50} approximately 20 and 30 μM). The reason for this variability is not clear and there was no obvious relationship in individual curves between the EC_{50} and the magnitude of the response to histamine. No similar variability was observed in either cerebellar or cerebral cortical slices.

To minimize potential problems in hippocampal slices in relating the response to the partial agonists to the maximum response to histamine, a higher standard dose of histamine (500 μM) was used in experiments with hippocampus than with cerebellum and cerebral cortex (200 μM) (see Methods). Thus even if the EC_{50} for histamine in hippocampal slices varied between 5 and 30 μM, the response to 500 μM histamine, as a percentage of the maximum, would only vary between 99 and 94%.

The best-fit values of the maximum responses to 2-methylhistamine, N^{α},N^{α}-dimethylhistamine and betahistine in hippocampus, expressed as a percentage of the histamine maximum, are set out in Table 2 and compared with the corresponding values in cerebellum and cerebral cortex. The order of effectiveness of the agonists, in terms of the maximum achievable response, is the same in hippocampus as in cerebellum and cerebral cortex, but the magnitude of the maximum response, relative to that of histamine, in hippocampus appears to be intermediate between the values in the other tissues (Table 2). The EC_{50} values for the agonists in hippocampus were nearer the values in cerebellum than cerebral cortex, although in the last region the errors tended to be larger as a consequence of the weaker responses. Thus the EC_{50} values for 2-methylhistamine were 12 ± 2, 18 ± 7 and 75 ± 15 μM in cerebellum, hippocampus and cerebral cortex, respectively, while the corresponding values for N^{α},N^{α}-dimethylhistamine were 16 ± 6, 16 ± 1 and 90 ± 44 μM.

Effect of Li^+ on the response to H_1-agonists in cerebellar slices

Differences in the characteristics of the histamine-induced accumulation of [^{3}H]-IP$_1$ between cerebellum and cerebral cortex are not limited to the characteristics of the dose-response curves. In cerebellar slices the reponse to histamine is notably less sensitive to the action of 10 mM Li^+ in enhancing [^{3}H]-IP$_1$ accumulation than in cerebral cortical slices (Daum *et al.*, 1984). To get some indication of whether the action of Li^+ might in some way contribute to the regional differences, measurements were made in cerebellar slices in the presence and absence of lithium. The particular advantage of the cerebellar slices in this respect is that a good response to histamine can be obtained in the absence of lithium (mean accumulation ± s.e.mean of

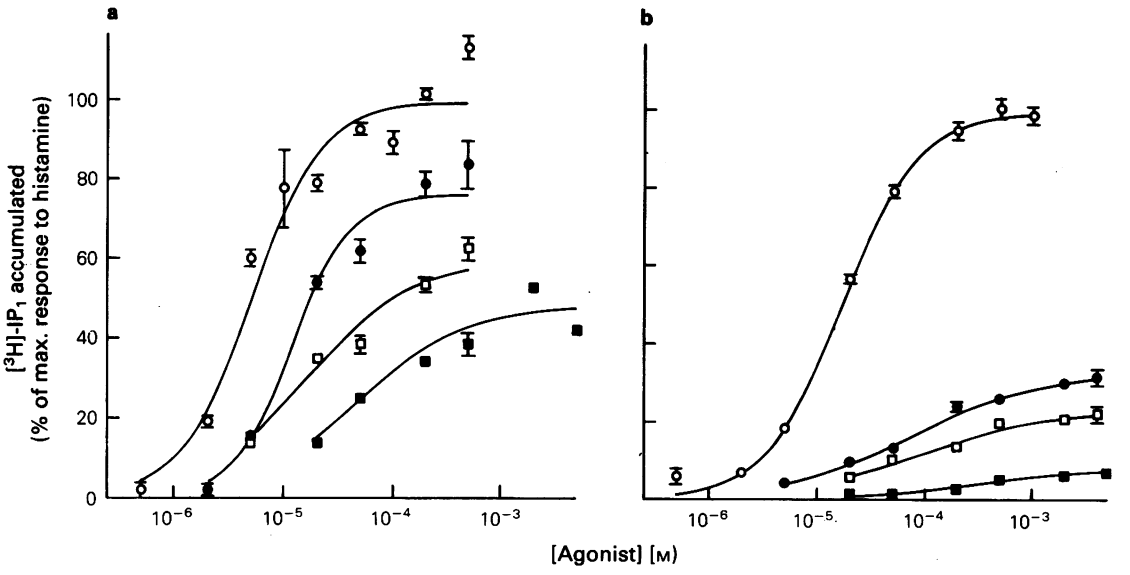

Figure 2 Comparison of responses to histamine H_1-agonists in (a) cerebellar and (b) cerebral cortical slices. Points for histamine are the combined values from a total of 9 experiments with cerebellum and 20 with cerebral cortex (see legend to Figure 1). Values for the other agonists are weighted means from 3 measurements of the dose-response curve (2 measurements for betahistine in cerebral cortex). The response to 200 μM histamine was measured in every experiment and used to relate the response with the agonist to the best-fit maximum response to histamine. Slices were exposed to the agonists for 60 min in the presence of 10 mM LiCl. (○) Histamine; (●) 2-methylhistamine; (□) N^{α},N^{α}-dimethylhistamine; (■) betahistine.

[^{3}H]-IP_1 induced by 200 μM histamine 7214 ± 1175 d.p.m., 9 experiments), but the response is still appreciably enhanced in the presence of 10 mM LiCl (18071 ± 1448 d.p.m., 21 experiments).

Dose-response curves for histamine and betahistine in the presence and absence of 10 mM Li^+, normalized by setting the best-fit maximum response to histamine = 100%, were superimposable. Similarly, the best-fit values of EC_{50} and the relative maximum responses for the other agonists did not differ significantly in the presence and absence of Li^+ (Table 3).

Inhibition of the histamine-induced response by mepyramine

The affinity constant for mepyramine, determined from inhibition of [^{3}H]-mepyramine binding, is closely similar in homogenates of cerebellum, $1.5 \times 10^9\,M^{-1}$, cerebral cortex, $1.4 \times 10^9\,M^{-1}$, and hippocampus, $1.0 \times 10^9\,M^{-1}$ (Aceves *et al.*, 1985). In agreement with this, mepyramine was approximately equiactive in inhibiting the accumulation of [^{3}H]-IP_1 induced by histamine in slices from the three regions. In cerebral cortical slices the affinity deduced from parallel shifts of the dose-response curve to histamine in 3 experiments with 5, 10 and 50 nM mepyramine was $4.6 \pm 1.0 \times 10^8\,M^{-1}$, whereas the mean affinity from 3 experiments with 10, 20 and 1000 nM mepyramine in cerebellar slices was $4.2 \times 0.6 \times 10^8\,M^{-1}$. A single experiment using hippocampal slices yielded an affinity for mepyramine of $5.5 \times 10^8\,M^{-1}$. All of these values are lower than the values from binding. However, 2 experiments in which measurements were made of the inhibition by increasing concentrations of mepyramine of the accumulation of [^{3}H]-IP_1 induced by a fixed concentration of histamine (50 μM in cerebellar slices, 1 mM in cerebral cortex) provided no evidence of secondary sites of histamine action. The best-fit values of the Hill coefficients were the same for both curves (1.31 ± 0.12 in cerebellum, 1.31 ± 0.17 in cerebral cortex) and the percentages of the response to histamine not sensitive to blockade by mepyramine, obtained from the same non-linear regression analysis, were 1.3 ± 0.8% and 5.3 ± 1.6% in cerebellum and cerebral cortex, respectively. The inhibition curve in cerebral cortex is shown in Figure 4. If the best-fit values of the EC_{50} for histamine in the two regions (see above) are taken to be the apparent dissociation constants, then the affinities for mepyramine derived from the IC_{50} values are $3.1 \times 10^8\,M^{-1}$ (cerebellum) and $3.5 \times 10^8\,M^{-1}$ (cerebral cortex).

Neither tetrodotoxin (1 μM) nor 5, 8, 11, 14-

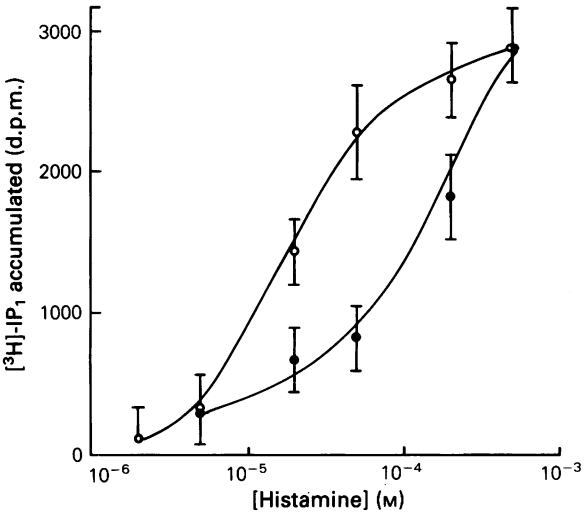

Figure 3 Inhibition by betahistine of histamine-induced accumulation of [^{3}H]-inositol 1-phosphate ([^{3}H]-IP_1) in cerebral cortical slices. Each point is the mean ± approximate s.e.mean of 3 determinations at each concentration of histamine in a single experiment. Basal accumulation was measured in the absence of both histamine and betahistine. Betahistine (1 mM) was added 30 min before the histamine, which was present for 60 min. The response to betahistine alone was 470 ± 223 d.p.m. (after subtraction of basal level). The curves shown were drawn by inspection. (○) Histamine alone; (●) Histamine + 1 mM betahistine.

eicosatetraynoic acid (ETYA) (15 μM) had any significant effect on the response to histamine in either cerebellar or cerebral cortical slices.

Histamine-induced inhibition of [^{3}H]-mepyramine binding

Histamine inhibited the binding of [^{3}H]-mepyramine to homogenates of guinea-pig cerebellum and cerebral cortex with apparent affinities of $3.7 \pm 0.3 \times 10^4$ (1 experiment) and $3.6 \pm 0.3 \times 10^4\ M^{-1}$ (weighted mean ± s.e. of 3 experiments), respectively (Hill coefficients 0.84 ± 0.04 and 0.79 ± 0.03). The similarity of the values in the two regions is in accord with the close similarity of the affinities for mepyramine (Aceves *et al.*, 1985).

Discussion

The results presented here show a clear difference in the positions of the dose-response curves for histamine-induced accumulation of [^{3}H]-IP_1 in cerebellar and cerebral cortical slices. No obvious differences in the EC_{50} values for histamine were apparent in our initial and more limited measurements in these regions (Daum *et al.*, 1984), but it is notable that the magnitude of the response to histamine in the current series of experiments, using a modified experimental protocol, is greater than in the earlier study. There seems little doubt that the difference between cerebellum and cerebral cortex is real and it is supported by the degree to which the other three H_1-agonists examined are partial agonists. In cerebellum, the tissue with the lower EC_{50} for histamine, all three agonists achieve a greater percentage of the maximum response to histamine than in cerebral cortex. In hippocampal slices the effectiveness of the agonists is intermediate between cerebral cortex and cerebellum, judging from the relative maximum responses, and argues that there are not just two discrete patterns of response, but rather that agonists can exhibit a spectrum of response in different tissues. Some caution has to be exercised in assessing the hippocampal

Table 2 Maximum responses to H_1-agonists as a percentage of that to histamine

	Maximum accumulation of [^{3}H]-IP_1 (% of histamine maximum)		
	Cerebellum	*Hippocampus*	*Cerebral cortex*
Histamine	100 ± 2	100 ± 3	100 ± 1
2-Methylhistamine	76 ± 5	52 ± 6	33 ± 2
N^{α},N^{α}-Dimethylhistamine	60 ± 7	39 ± 2	24 ± 3
Betahistine	48 ± 6	20 ± 3	8 ± 1

Best-fit values of the maximum accumulation of [^{3}H]-inositol 1-phosphate ([^{3}H]-IP_1) ± estimated s.e. were obtained from non-linear regression analysis (see Methods) of the combined data from 3 experiments with each agonist in each region (4 for N^{α},N^{α}-dimethylhistamine in hippocampus, 2 for betahistine in cerebral cortex). The response to 200 μM histamine (cerebellum and cerebral cortex) or 500 μM histamine (hippocampus) was measured in each experiment and used to relate the response to the agonist to the maximum response to histamine (see the legends to Figures 1 and 2).

Table 3 Parameters of histamine H_1-agonist stimulated accumulation of [^{3}H]-inositol 1-phosphate ([^{3}H]-IP_1) in cerebellar slices in the presence and absence of Li^+.

	EC_{50} (μM)		Maximum response (% of histamine max)	
	$+Li^+$	$-Li^+$	$+Li^+$	$-Li^+$
Histamine	5 ± 1 (9)	4 ± 1(3)	100	100
2-Methylhistamine	12 ± 2 (3)	15 ± 3(1)	76 ± 5	71 ± 4
N^{α},N^{α}-Dimethylhistamine	16 ± 6 (3)	19 ± 3(1)	60 ± 7	66 ± 3
Betahistine	48 ± 14(3)	42 ± 9(2)	48 ± 6	43 ± 3

Best-fit values ± estimated s.e. of EC_{50}, the concentration of agonist giving the half-maximal accumulation of [^{3}H]-IP_1, and the maximum response were obtained by fitting concentration-response data to a Hill equation using non-linear regression analysis as described under Methods. Incubation time with agonist was 60 min. The agonist-induced accumulation was defined as accumulation of [^{3}H]-IP_1 in presence of agonist minus the basal level. The response to 200 μM histamine was measured in all experiments with the other H_1-agonists and the value obtained used to relate the response to the agonist to the maximum response to histamine measured under the same conditions, i.e. with or without 10 mM LiCl present. The number of experiments with each agonist is given in parentheses.

data, since the histamine dose-response curve does show a degree of variability not observed in either cerebellum or cerebral cortex. Nonetheless, the choice of a relatively high concentration of histamine, 500 μM, for relating the responses of other agonists to that of histamine makes it unlikely that any variation in the EC_{50} for histamine could produce a correction factor big enough to make the hippocampal data (Table 2) match that of either of the other two regions.

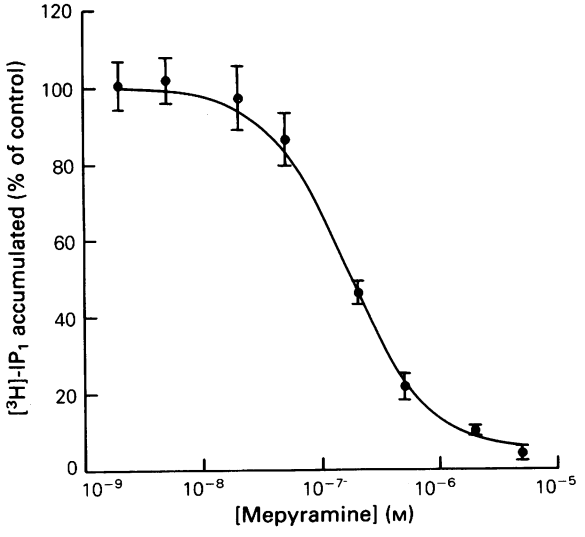

Figure 4 Inhibition by mepyramine of histamine-induced accumulation of [^{3}H]-inositol 1-phosphate ([^{3}H]-IP_1) in cerebral cortical slices. Points are the means ± approximate s.e.mean of the ratio: histamine-induced accumulation in the presence of mepyramine × 100/histamine-induced accumulation in the absence of mepyramine. Quadruplicate determinations were made at each concentration of antagonist. Mepyramine was present for 30 min before addition of 1 mM histamine. Incubation with histamine was for 60 min. The curve drawn is a best-fit line obtained by weighted non-linear regression analysis as described under Methods.

The implication of these observations is that at least in cerebellum and hippocampus the accumulation of [^{3}H]-IP_1 induced by histamine in lithium-treated tissue and measured over an extended incubation period, here 60 min, is not as directly related to agonist-receptor binding as the simplest schemes (agonist binding → phosphatidylinositol, 4, 5-bisphosphate (PIP_2) hydrolysis → IP_3 → IP_2 → IP_1) would suggest. At least one of the implicit assumptions made must need qualification, although the experiments described here give no clear indication which that might be. It seems unlikely that it is a result of the presence of 10 mM Li^+. There are regional differences in the sensitivity of histamine-induced accumulation of [^{3}H]-IP_1 to the potentiating effects of 10 mM Li^+ (Daum *et al.*, 1984) and the lesser sensitivity of cerebellar slices to Li^+ has been confirmed with the present experimental protocol. However, the lack of effect of Li^+ on the characteristics of the dose-response curves to H_1-agonists in cerebellar slices rules out any direct effect of Li^+ and makes it unlikely that the variation in response between regions is some consequence of differing levels of inositol 1-phosphatase activity, since the presence of 10 mM Li^+ in cerebellar slices does reduce the rate of breakdown of [^{3}H]-IP_1 (data not shown).

One explanation for the apparent complexity of the response to histamine is that there is more than one pathway for IP_1 formation in response to H_1-agonists. There is now good evidence for the presence of higher inositol phosphates in certain tissues, particularly IP_4

(Batty *et al.*, 1985), and both the 1,4,5- and 1,3,4-isomers of IP_3 have been detected in the same tissue following agonist stimulation (Irvine *et al.*, 1984; Burgess *et al.*, 1985). The relationship between these various phosphates remains unclear, but if the 1,4,5- and 1,3,4-isomers arise from different pathways of agonist-induced phosphoinositide breakdown, then determining [^{3}H]-IP_1 after long incubation periods may be misleading. The anion-exchange assay procedure we have used does not separate the isomers of IP_3, but we have failed to detect any significant amounts of [^{3}H]-IP_4. Another important possibility which we cannot rule out is that some [^{3}H]-IP_1 may be formed directly from the breakdown of phosphatidylinositol (PI), rather than PIP_2, such as appears to occur as a secondary response in thrombin-stimulated platelets (Wilson *et al.*, 1985). A similar effect secondary to H_1-receptor activation and which varied in extent between tissues could explain the observed regional differences. It may be noted that the Hill coefficients for histamine action are consistently greater than unity in both cerebellum and cerebral cortex, although the values do not differ significantly between the two regions.

The similar values obtained for the affinity constant for mepyramine in cerebellum and cerebral cortex (and in one experiment in hippocampus) argue against any obvious differences in the extent of H_1-receptor involvement in histamine-induced [^{3}H]-IP_1 accumulation or in the H_1-receptors properties. It is nonetheless notable that the affinities for mepyramine are lower by a factor of approximately three than the corresponding affinity constants determined from inhibition of [^{3}H]-mepyramine binding in the three regions (Aceves *et al.*, 1985) or in our earlier measurements against inositol phospholipid breakdown (Daum *et al.*, 1984). This raises the possibility of a non-H_1-receptor-mediated component of the response, but it is apparently present to the same extent in cerebellum, cerebral cortex and hippocampus. The observations of Donaldson & Hill (1985) on the properties of the response to histamine in guinea-pig intestinal smooth muscle, which is notably insensitive to H_1-receptor blockade, give some credence to this possibility, although they obtained a value for the affinity constant of mepyramine in cerebellar slices of 9×10^8 M^{-1}. However, if there is a second component in the response to histamine, then it might have been expected that it would be apparent in the curves of the inhibition of the response to histamine by increasing concentrations of mepyramine. These curves show no evidence of a second component, but it is interesting that the Hill coefficients appear to be greater than unity. This might indicate that there are problems in achieving a true equilibrium between the concentrations of antagonist in the bathing solution and in the intercellular spaces in the slices.

Perhaps the simplest explanation for the differences in the characteristics of the response to the agonists is that there is a receptor reserve (spare receptors) for agonists of high efficacy which differs in extent in different brain regions. The relative effectiveness of the H_1 partial agonists between cerebellum and cerebral cortex could be readily explained in this way. A similar explanation has been proposed for differences in the muscarinic agonist-induced accumulation of [^{3}H]-IP_1 between slices of cerebral cortex and parotid gland of the rat (Jacobson *et al.*, 1985) and between regions of rat brain (Fisher & Bartus, 1985). This apparent receptor reserve might be either a consequence of secondary responses, as discussed above, or might turn on the presence of a GTP-binding protein, which effects the coupling between receptor and PIP_2-specific phospholipase C. There is growing evidence for the presence of such a protein (see e.g. Berridge & Irvine, 1984) and if the receptors are mobile in the membrane phase, then differences in receptor number could influence the rate of formation of an agonist-receptor-GTP protein complex. However, it is not certain that all the observations are consistent with the spare receptor explanation. The value for the EC_{50} for histamine in hippocampus might have been expected to be intermediate between the values in cerebellum and cerebral cortex, although there are reservations about the hippocampal value. Similarly the EC_{50} values for the partial agonists in the three regions do not seem entirely consistent with the spare receptor explanation, but there are appreciable errors on the values in cerebral cortex and until a greater degree of accuracy can be achieved it will not be possible to make a more certain evaluation. It may also be noted that studies with phenoxybenzamine have failed to yield any evidence for a receptor reserve for α_1-adrenoceptor-mediated accumulation of [^{3}H]-IP_1 in rat cerebral cortex (Kendall *et al.*, 1985). In addition very recently Claro *et al.* (1986) have provided similar evidence for a lack of any receptor reserve to histamine in the same tissue, although the EC_{50} for histamine, 95 μM, was much higher than in any of the guinea-pig regions we have studied.

In summary, the results of this study reflect both the utility and the limitations of histamine H_1-agonist-induced phosphoinositide breakdown. The utility of agonist-induced accumulation of [^{3}H]-IP_1 in Li^+-treated tissue as a general reaction reflecting the presence of functional H_1-receptors is apparent. Also, the agreement between the characteristics of 2-methylhistamine, N^α,N^α-dimethylhistamine and betahistine as partial agonists in stimulating [^{3}H]-IP_1 accumulation and their behaviour as partial agonists in potentiating adenosine-stimulated cyclic AMP accumulation in guinea-pig cerebral cortical slices (Daum *et al.*, 1982) is pleasing. However, it is clear that a much more detailed investigation of the pathways of histamine

H_1-agonist-induced phosphoinositide breakdown will be necessary before the full utility of this response as a mirror of agonist-receptor interaction can be assessed.

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