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Differential effect of temperature on histamine- and carbachol-stimulated inositol phospholipid breakdown in slices of guinea-pig cerebral cortex

Heather Carswell, A.G. Galione[1] & J.M. Young

Department of Pharmacology, University of Cambridge, Hills Road, Cambridge, CB2 2QD

1 Slices of guinea-pig cerebral cortex were incubated with [^{3}H]-inositol at 37°C before exposure to histamine or carbachol at 37°C or 25°C. Histamine-stimulated accumulation of [^{3}H]-inositol 1-phosphate ([^{3}H]-IP_1) at 25°C was only 5–7% of that at 37°C, whereas for carbachol the response at 25°C was 45–49% of that at 37°C.

2 The affinity of benzilylcholine, obtained from inhibition of carbachol-induced accumulation of [^{3}H]-IP_1 was similar at 25°C and 37°C, but the EC_{50} for carbachol was lower at 25°C (20 ± 2 μM) than at 37°C (42 ± 2 μM).

3 The IC_{50} for histamine inhibition of [^{3}H]-mepyramine binding to homogenates of guinea-pig cerebral cortex did not differ significantly at 25°C and 37°C.

4 Histamine-induced accumulations of [^{3}H]-IP_2 and [^{3}H]-IP_3 at 25°C, expressed as a percentage of the accumulation at 37°C, were also much less than the corresponding value for carbachol.

5 These observations imply that the locus or pathway(s) of agonist-induced formation of [^{3}H]-IP_1 are not the same for histamine and carbachol.

Introduction

There is now a wealth of evidence that receptor-coupled inositol phospholipid breakdown, may be an early step in the chain of events which leads to the cellular response to 'calcium-mobilising' agonists (Michell *et al.*, 1981; Berridge, 1984; Berridge & Irvine, 1984). In most tissues the agonist-catalysed step appears to be the hydrolysis of phosphatidylinositol 4,5-bisphosphate, but in some cells, such as thrombin-stimulated platelets (Wilson *et al.*, 1985), other mechanisms may contribute to the formation of inositol 1-phosphate (IP_1). Whether such secondary mechanisms also operate in cells of the mammalian CNS is not established, but there are features of the accumulation of IP_1 induced by histamine in lithium-treated slices of guinea-pig brain which suggest that the actions of histamine may be more complex than the simple reaction schemes would suggest (Carswell *et al.*, 1985; Carswell & Young, 1985). The same may be true in slices of guinea-pig intestinal smooth muscle, where the response to histamine is relatively insensitive to histamine H_1-antagonists (Donaldson & Hill, 1985).

In the course of an investigation of the properties of histamine-induced accumulation of IP_1 in slices of guinea-pig cerebral cortex, we observed that lowering the temperature to 25°C appeared to have a much greater depressive effect on the response than would have been expected from measurements on other systems, such as thyrotropin releasing hormone (TRH)-stimulated GH_3 pituitary tumour cells (Drummond *et al.*, 1984). To try and establish whether the sensitivity of the histamine response is unusual, we have compared the effect of temperature on the responses to histamine and to the muscarinic agonist carbachol in slices of guinea-pig cerebral cortex. Some of these results have been presented in preliminary form to the British Pharmacological Society (Carswell *et al.*, 1986).

[1] Present address: AFRC Unit of Insect Neurophysiology and Pharmacology, Department of Zoology, University of Cambridge, Downing Street, Cambridge, CB2 3EJ.

Methods

Agonist-induced accumulation of [^{3}H]-inositol phosphates

Cross-chopped slices (350 × 350 μm, McIlwain tissue chopper) of guinea-pig (Dunkin-Hartley strain, males) cerebral cortex were washed three times and then incubated at 37°C for 60 min in Kreb-Henseleit medium (in mM: NaCl 116, KCl 4.7, $MgSO_4$ 1.2, KH_2PO_4 1.2, $HaHCO_3$ 25, $CaCl_2$ 2.5 and D-glucose 11) with three further changes of medium. The medium was bubbled throughout with O_2/CO_2 (95:5, vol/vol). The slices were then transferred to Krebs-Henseleit solution (8.4 ml per cortex) containing 0.33 μM *myo*-[^{3}H]-inositol and incubated for a further 30 min at 37°C. The slices were washed three times and then transferred to a flat-bottomed vial (Hughes & Hughes Ltd, scintillation vial insert) and allowed to settle under gravity. Aliquots of the slices (40 μl) were added to 200 μl Krebs-Henseleit medium containing 10 mM LiCl and 1 mM unlabelled *myo*-inositol and incubated for 15 min at 25°C or 37°C. At the end of this time histamine or carbachol (10 μl of the appropriate solution) was added and the incubation continued for the desired period before termination by addition of 0.94 ml chloroform/methanol (1:2, vol/vol) or 200 μl ice-cold 15% trichloroacetic acid.

This procedure, in which the [^{3}H]-inositol is removed before addition of the agonist + excess unlabelled inositol, is termed 'pulse-labelling'. In a few experiments, denoted as 'continuous labelling', the [^{3}H]-inositol was not removed following the 30 min incubation at 37°C and no unlabelled inositol was present during the period of exposure to the agonist. Otherwise the procedure was the same as described above.

In incubations terminated by addition of chloroform/methanol, [^{3}H]-inositol phosphates were extracted and separated essentially as described by Berridge *et al.* (1982). 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 then washed with 5 ml water to remove any [^{3}H]-inositol, followed by 8 ml 60 mM ammonium formate/5 mM sodium tetraborate to remove [^{3}H]-glycerophosphoinositol. [^{3}H]-IP_1 was then eluted with 8 ml 200 mM ammonium formate/100 mM formic acid. In some experiments [^{3}H]-IP_2 and [^{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. Aquasol-2 (8 ml) was added to each fraction and tritium determined by scintillation counting.

In incubations terminated by addition of trichloroacetic acid the mixture was allowed to stand on ice for 15 min before centrifugation at 950 *g* for 5 min. A sample of the supernatant (250 μl) was extracted 5 times with ether to remove trichloroacetic acid and the aqueous layer then neutralised with Na tetraborate before separation of the [^{3}H]-inositol phosphates as described above.

Inhibition of [^{3}H]-mepyramine and [^{3}H]-methylscopolamine binding

Preparation of a membrance fraction from guinea-pig cerebral cortex and measurement of histamine inhibition of [^{3}H]-mepyramine binding in 50 mM Na-K phosphate buffer, pH 7.5, was carried out essentially as described previously (Aceves *et al.*, 1985), except that incubations were for 30 min at 37°C or 60 min at 25°C. The concentration of [^{3}H]-mepyramine was 0.37–0.57 nM and non-specific binding was defined with 2 μM promethazine. Pentuplicate measurements were made at 12–14 histamine concentrations.

Measurements of benzilylcholine inhibition of [^{3}H]-N-methylscopolamine binding were made similarly, with non-specific binding defined by 1 μM N-methylatropine.

Analysis of data

Concentration-response curves for histamine- and carbachol-induced accumulation of [^{3}H]-IP_1 following 60 min incubation with the agonist were constructed by combining the data (after subtraction of the accumulation in the absence of agonist) from two or more experiments. The response to 200 μM histamine or 1 mM carbachol was measured in all experiments and was used to correct for differences in the absolute level of accumulated [^{3}H]-IP_1 between slice preparations. The concentration-response curves 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 = $Resp_{max} \times D^n/(D^n + EC_{50}{}^n)$, where D is the agonist concentration, n is the Hill coefficient, EC_{50} is the concentration giving 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 affinity constant of benzilylcholine against carbachol-induced [^{3}H]-IP_1 accumulation was obtained from parallel shifts of the concentration-response curve to carbachol, using the relationship: Concentration-ratio = $[A] \times K_A + 1$, where [A] is the concentration of benzilylcholine and K_a the affinity constant. The concentration-ratio is the concentration of carbachol required for a given response in the presence of antagonist divided by the concentration required in the absence of antagonist.

Curves of the inhibition by histamine 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 histamine required for 50% inhibition of the histamine-sensitive component of the response) and the percentage of the response insensitive to inhibition by histamine 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 $mmol^{-1}$) and (−)-[N-methyl-^{3}H]-N-methylscopolamine chloride (76 Ci $mmol^{-1}$) from Amersham International. Histamine dihydrochloride and carbachol (carbamylcholine chloride) were obtained from Sigma. Benzilylcholine chloride was prepared by the method of Ford-Moore & Ing (1947).

Results

Time-course and characteristics of histamine- and carbachol-stimulated [^{3}H]-inositol 1-phosphate accumulation

The time courses of histamine- and carbachol-stimulated accumulation of [^{3}H]-IP_1 in cerebral cortical slices prelabelled with [^{3}H]-inositol were similar to those described previously for histamine using the continuous labelling protocol (Daum *et al.*, 1984; Carswell *et al.*, 1985). The agonist-induced [^{3}H]-IP_1 accumulation increased approximately linearly with time, while the basal accumulation showed no significant change over the same period (5–90 min). The level of the basal accumulation was somewhat lower in experiments using the pulse-labelling protocol, 772 ± 61 d.p.m. (mean ± s.e.mean from 11 determinations), than in experiments carried out under continuous labelling conditions, 1032 ± 171 d.p.m. (34 experiments).

The EC_{50} and Hill coefficients for concentration-response curves for the accumulation of [^{3}H]-IP_1 induced by histamine and carbachol at 37°C were closely similar for both experimental protocols (Table 1). The pulse-labelling protocol was used in all subsequent experiments.

Agonist-induced [^{3}H]-inositol 1-phosphate formation at 37°C and 25°C

The response to carbachol was decreased when cerebral cortical slices, prelabelled at 37°C, were incubated with the agonist at 25°C rather than 37°C. The time-course of the response to 0.1 mM carbachol at 25°C is shown in Figure 1(a) and comparison made with the basal and agonist-stimulated levels measured at 45 min at 37°C on the same slice preparation in the same experiment. Essentially similiar results were obtained in a second independent experiment. The basal accumulation was not significantly reduced at the lower temperature, 578 ± 126 d.p.m. at 25°C (mean ± s.e.mean of 5 determinations) and 772 ± 61 at 37°C (11 determinations).

The response to 0.2 mM histamine was reduced to a much greater extent than that to carbachol on lower-

Table 1 Parameters of concentration-response curves for histamine and carbachol-induced accumulation of [^{3}H]-inositol 1-phosphate ([^{3}H]-IP_1)

[^{3}H]-inositol labelling	Histamine EC_{50} (μM)	Histamine n_H	Carbachol EC_{50} (μM)	Carbachol n_H
37°C				
Continuous	16 ± 1	1.24 ± 0.03 (20)	42 ± 4	0.95 ± 0.05 (3)
Pulse	17 ± 1	1.21 ± 0.07 (2)	42 ± 4	1.02 ± 0.04 (2)
25°C				
Continuous		—	25 ± 2	0.91 ± 0.03 (2)
Pulse		—	20 ± 2	0.99 ± 0.06 (2)

Values are the best-fit parameters ± estimated s.e. obtained from non-linear regression analysis of concentration-response curves measured using pulse- or continuous-labelling conditions as described under Methods. The points fitted were the weighted means at each concentration from the number of independent experiments given in parentheses. n_H is the Hill coefficient. The values for histamine at 37°C under continuous-labelling conditions are taken from a series of experiments using a simplified protocol in which the labelling with [^{3}H]-inositol and incubation with histamine took place in the final incubation vial (Daum *et al.*, 1984; Carswell *et al.*, 1985).

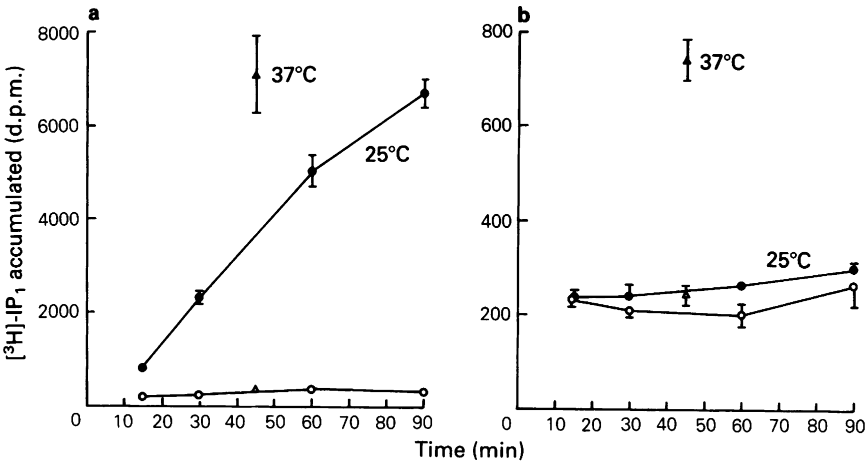

Figure 1 Time-course of carbachol- and histamine-induced accumulation of [^{3}H]-inositol 1-phosphate ([^{3}H]-IP$_1$) at 25°C. (a) Carbachol, (b) histamine. Slices of guinea-pig cerebral cortex were labelled with [^{3}H]-inositol before incubation with 0.1 mM carbachol or 0.2 M histamine at 25°C or 37°C (45 min time point), as described under Methods. The data in (a) and (b) are from independent experiments but for each agonist, measurements were made at 25°C and 37°C with the same slice preparation at the same time. Points are the mean ± s.e.mean of 5 replicate determinations at each time point at 25°C and 6 determinations at 37°C. Where no error bars are shown the error was within the size of the symbol. (●), 25°C in presence of agonist; (O), 25°C no agonist; (▲), 37°C in presence of agonist; (△), 37°C no agonist.

ing the incubation temperature to 25°C. An experiment carried out under the same conditions as for carbachol is shown in Figure 1(b). Even after 90 min exposure to histamine only a very small accumulation of [^{3}H]-IP$_1$ was detected. Similar results were obtained in two further experiments.

The marked difference of the effect of temperature on the response to histamine and carbachol might reflect changes at the level of the agonist-receptor interaction rather than in the receptor-coupled breakdown. The affinity of benzilylcholine, a muscarinic antagonist, deduced from the parallel shift of the concentration-response curve for carbachol-induced [^{3}H]-IP$_1$ accumulation in the presence of 50 nM benzilylcholine, was little altered by the decrease in temperature, K_a 3.3×10^8 M^{-1} at 37°C and 3.7×10^8 M^{-1} at 25°C, in good agreement with the value of $3.4 \pm 0.3 \times 10^8$ M^{-1} obtained from measurements of the inhibition by benzilylcholine of the binding of [^{3}H]-N-methylscopolamine to a homogenate of the same tissue at 30°C. These values are in accord with the affinity reported for inhibition of carbachol-induced contraction of guinea-pig ileum at 37°C, 3.2×10^8 M^{-1} (Abramson *et al.*, 1969). However, in contrast to the apparent lack of change in the affinity of the muscarinic antagonist, the EC$_{50}$ for carbachol-stimulated accumulation of [^{3}H]-IP$_1$ was lower at 25°C than at 37°C (Table 1). A similar value was measured under continuous labelling conditions (Table 1).

The very small accumulation of [^{3}H]-IP$_1$ induced by histamine at 25°C made it impracticable to use this response to test for changes in agonist-receptor interaction on lowering the temparature from 37°C to 25°C. To try and assess the likelihood of such changes, the characteristics of histamine inhibition of the binding of [^{3}H]-mepyramine to homogenates of guinea-pig cerebral cortex and cerebellum were determined at 37°C and 25°C (Table 2). The similarity of the values of the IC$_{50}$, corrected to allow for competition with the ^{3}H-ligand (apparent K_d values), gives no indication of large changes in the nature of the interaction of histamine with the H$_1$-receptor as the temperature is lowered to 25°C.

Assuming that the affinity for histamine remains unaltered and that the affinity for carbachol increases as indicated by the decrease in the EC$_{50}$ (Table 1), then the maximum response to each agonist at 25°C can be calculated as a percentage of the maximum response at 37°C. The values obtained from experiments such as those shown in Figure 1, in which measurements at both temperatures were made at the same time with the same slice preparation, are set out in Table 3 (Exptl series 1). Each value is from an independent experiment with 0.2 mM histamine or 0.1 mM carbachol and an incubation time of 45 min.

Table 2 Parameters of histamine inhibition of [^{3}H]-mepyramine binding to cerebral cortical homogenates at 37°C and 25°C

37°C		*25°C*	
IC_{50} corr. (μM)	n_H	*IC_{50} corr.* (μM)	n_H
25 ± 5	(0.77 ± 0.08)	30 ± 3	(0.97 ± 0.08)
31 ± 2	(0.86 ± 0.05)	29 ± 2	(0.94 ± 0.05)
26 ± 2	(0.75 ± 0.03)	30 ± 2	(0.88 ± 0.05)

Values are best-fit parameters ± estimated s.e. obtained from non-linear regression analysis of curves of inhibition of 0.37–0.57 nM [^{3}H]-mepyramine binding to a membrane fraction from guinea-pig cerebral cortex as described under Methods. IC_{50} corr. is the concentration of histamine required for 50% inhibition of the histamine-sensitive binding, corrected for competition with [^{3}H]-mepyramine, i.e. the apparent equilibrium dissociation constant. n_H is the Hill coefficient. Each pair of values of IC_{50} corr. and n_H is from an independent experiment.

To minimise the apparent change in the occupancy of carbachol between the two temperatures a second series of measurements was made with a higher concentration of carbachol, 1 mM. At this concentration the increase of occupancy at 25°C over that at 37°C is very small (0.98 at 25°C, 0.96 at 37°C). In each experiment measurements were made of the responses to both histamine and carbachol at both 37°C and 25°C. The calculated maximum response to each agonist, expressed as a percentage of the maximum response at 37°C, are set out in Table 3 (Exptl series 2). The much greater reduction of the response to histamine on lowering the temperature is again clear.

Effect of temperature on agonist-induced formation of [^{3}H]-IP_2 and [^{3}H]-IP_3

The much reduced histamine-induced accumulation of [^{3}H]-IP_1 at 25°C does not appear to be a consequence of a shift in the proportions of [^{3}H]-IP_1, [^{3}H]-IP_2 and [^{3}H]-IP_3 produced. The amounts of [^{3}H]-IP_2 and [^{3}H]-IP_3 produced following a 60 min incubation with agonist were much smaller than the amount of [^{3}H]-IP_1, consistent with the lesser inhibitory effect of 10 mM Li^+ on the phosphatases which hydrolyse IP_2 and IP_3. Thus the mean calculated maximum accumulations of [^{3}H]-IP_2 and [^{3}H]-IP_3 induced by carbachol at 37°C in the 5 experiments in which they were measured were 1827 ± 502 and 993 ± 260 d.p.m., respectively (basal levels subtracted). The large errors indicate the appreciable variation between experiments of the absolute amounts of the phosphates generated, as was also observed for [^{3}H]-IP_1, 17,065 ± 2527 d.p.m. (mean ± s.e.mean from 7 determinations). Thus the amounts of [^{3}H]-IP_2 and [^{3}H]-IP_3 measured at 37°C were 11% and 2%, respectively, of that of [^{3}H]-IP_1. At 25°C the amounts of [^{3}H]-IP_2 and [^{3}H]-IP_3 produced by carbachol were reduced to a similar extent so that the maximum accumulations at 25°C as a percentage of the maximum at 37°C were

Table 3 Agonist-induced [^{3}H]-inositol 1-phosphate ([^{3}H]-IP_1) accumulation at 25°C as a percentage of that at 37°C

	Max. [^{3}H]-IP_1 accumulation at 25°C × 100 / Max. [^{3}H]-IP_1 accumulation at 37°C	
	Histamine	*Carbachol*
Exptl series 1		
	6	44
	9	68
	5*	43*
	10*	26*
Mean + s.e.mean	7 ± 1	45 ± 9
Exptl series 2		
	4	44
	8	45
	2*	57*
Mean + s.e.mean	5 ± 2	49 ± 5

The accumulations of [^{3}H]-IP_1 induced by histamine and carbachol were measured as described under Methods. In experimental series 1, incubations were with 0.2 mM histamine or 0.1 mM carbachol for 45 min. In series 2, 0.2 mM histamine and 1 mM carbachol were present for 60 min. For carbachol, maximum accumulations of [^{3}H]-IP_1 at each temperature were calculated from the values measured, taking the ED_{50} to be equal to the equilibrium dissociation constant, i.e. 42 μM at 37°C and 20 μM at 25°C (Table 1). The affinity of histamine was assumed not to change significantly. The accumulation of [^{3}H]-IP_1 induced by histamine or carbachol was always measured at the two temperatures in the same experiment with the same slice preparation. In series 1, the response to histamine and carbachol was measured in independent experiments. In series 2, responses to both agonists were measured in a single experiment, i.e. the values shown are 3 paired determinations. The values marked * were obtained after extraction of [^{3}H]-IP_1 with trichloracetic acid/ether rather than chloroform/methanol (see Methods).

60 ± 12% and 59 ± 9%, respectively. These percentages compare with the two values of 45 ± 9% and 49 ± 5% measured for [^{3}H]-IP_3 (Table 3). In all of these experiments the responses at 37°C and 25°C were measured in the same experiment with the same slice preparation.

The amounts of [^{3}H]-IP_2 and [^{3}H]-IP_3 produced by histamine were smaller than those following carbachol, but the percentages of the maximum [^{3}H]-IP_1 response to histamine at 37°C (4960 ± 545 d.p.m., 6 determinations) were similar, 14% and 3%, respectively, to those observed with carbachol, 11% and 2%. The formation of both [^{3}H]-IP_2 and [^{3}H]-IP_3 in response to histamine was markedly depressed at 25°C so that the errors on the measurements were large. The amount of [^{3}H]-IP_3 measured at 25°C was too small (52 ± 42 d.p.m., 5 determinations) to allow meaningful comparison with the accumulation at 37°C. The calculated maximum induced accumulation of [^{3}H]-IP_2 at 25°C was only 14 ± 6% of that at 37°C (cf. 60 ± 12% for carbachol). The results were similar whether the inositol phosphates were extracted using the chloroform/methanol or trichloracetic acid/ether method.

Discussion

A decrease in the rate of biochemical reactions as the temperature is lowered is the rule. However, the extent of the decrease in histamine-induced accumulation of [^{3}H]-IP_1 as the temperature is lowered from 37°C to 25°C is much greater than might have been expected and contrasts with the lesser decrease in the response to carbachol. The latter is more in line with the fall in the response to TRH in GH_3 pituitary tumour cells over the same temperature range (Drummond *et al.*, 1984). The apparent implication is that the locus or pathway of the histamine-induced accumulation differs from that induced by carbachol. The other possibility, that there is an effect of temperature at the level of the histamine-receptor interaction, rather than the subsequent biochemical events, looks unlikely, but cannot be ruled out completely.

Possible changes in histamine receptor conformation and function with temperature have been the subject of some debate (summarised by Cook *et al.*, 1985). An earlier suggestion of a temperature-dependent interconversion of H_1- and H_2-receptors has not been substantiated by subsequent experimental work (Bertaccini & Zappia, 1983; Cook *et al.*, 1985) and there is no marked change in the affinity of [^{3}H]-mepyramine binding to H_1-receptors between 37°C and 25°C, although the rate constants do decrease (Wallace and Young, 1983). However, the rate constants for [^{3}H]-quinuclidinyl benzilate, a muscarinic antagonist, also change markedly with temperature (Gorissen *et al.*, 1978; Hurko, 1978) so that an effect on rate constants alone seems unlikely to be the basis of the differential effect of temperature on histamine- and carbachol-induced [^{3}H]-IP_1 accumulation. Evidence based on antagonist affinities is in any case limited in value, since antagonist binding is not necessarily a reliable guide to changes in agonist function, as the data for carbachol indicate. The affinity of benzilylcholine does not change significantly between 37°C and 25°C, but the EC_{50} for carbachol is significantly lower ($P < 0.01$) at the lower temperature (Table 1). The very small accumulation of [^{3}H]-IP_1 induced by histamine at 25°C has made it impracticable to determine whether there are also changes in the EC_{50} for histamine. However, if the marked temperature sensitivity is to be explained in this way, the change in the EC_{50} for histamine would have to be considerable, from 16 μM at 37°C to around 1.6 mM at 25°C. This is possible, but seems improbable. The corrected IC_{50} (apparent K_d) for histamine inhibition of [^{3}H]-mepyramine binding does not differ significantly at the two temperatures (Table 2), but the difficulty here is that it is uncertain how agonist binding is to be related to agonist function. Indeed, one of the objects of our studies of histamine-induced IP_1 formation is to establish whether this simple assay is suitable for correlating agonist binding and agonist function. The lack of any marked change in the contractile response of the guinea-pig ileum and guinea-pig colon to histamine between 37°C and 25°C (Cook *et al.*, 1985) argues against any universal and marked effect of temperature on H_1-receptor function. However, it is not clear how histamine-induced inositol phospholipid breakdown in the guinea-pig ileum is to be related to histamine-induced contraction (Donaldson & Hill, 1985) and the possibility must be borne in mind that there could be differences in the coupling of central and peripheral H_1-receptors with their effectors (Harrison *et al.*, 1984).

The most likely explanation for the differential temperature sensitivity of the histamine- and carbachol-induced accumulations of [^{3}H]-IP_1 is that the locus or pathways of the responses differ. This explanation is supported indirectly by reports of other differences between histamine and carbachol responses in which temperature is not a factor. Histamine (H_1), but not carbachol, potentiates the accumulation of cyclic AMP induced by 2-chloroadenosine in guinea-pig cerebral cortical slices (Hollingsworth & Daly, 1985) and, conversely, adenosine analogue have been reported to enhance histamine-induced, but not carbachol-induced, accumulation of inositol phosphates in the same tissues (Hollingsworth *et al.*, 1986). In rat hippocampal slices, K^+ facilitates IP_1 accumulation induced by carbachol, but not by histamine (Eva & Costa, 1986).

The biochemical basis of these differences is not known. The magnitude of the response to carbachol at 37°C is greater than that to histamine, but there is no indication that the difference is some way related to this, since the effect of temperature on the carbachol response was very similar in experiments with 0.1 mM (Table 3, Exptl series 1) and 1 mM carbachol (Series 2). Nor does temperature obviously change the relative amounts of IP_1, IP_2 and IP_3, although it must be borne in mind that the assay does not separate the isomers of the bis- or tris-phosphates. However, there are now several pieces of indirect evidence that the histamine-induced accumulation of $[^3H]$-IP_1 may not be a simple sequence $PIP_2 \rightarrow IP_3 \rightarrow IP_2 \rightarrow IP_1$. The time-course of histamine-induced $[^3H]$-IP_1 accumulation seems to be characterized by a lag period (Daum *et al.*, 1984; Carswell *et al.*, 1985), the response is partly Ca^{2+}-dependent (Carswell *et al.*, 1985) and concentration-response curves for histamine consistently have Hill coefficients > 1 (Table 1). This last observation contrasts with the near hyperbolic curves for carbachol (Table 1). None of these observations or the differential temperature sensitivity is alone convincing evidence of a complex response, but put together they are suggestive. What is clear is that the simple assay of histamine-induced $[^3H]$-IP_1 accumulation may not reflect the agonist-receptor interaction as closely as had been hoped. The major assumption is that as long as H_1-agonist-induced phosphoinositide hydrolysis leads eventually to IP_1, the breakdown of which is inhibited by Li^+, then for every molecule of PIP_2 hydrolysed, one molecule of IP_1 will be formed, irrespective of whether 1, 3, 4-trisphosphate is also formed (Irvine *et al.*, 1986), provided that the incubation period is sufficiently long so that the amounts of IP_2 and IP_3 present are relatively small. There must be some doubt whether this assumption is justified. Whether other pathways of IP_1 formation, such as agonist-stimulated hydrolysis of PI itself, are involved will need to be part of a more detailed examination of the response to histamine.

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