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Studies on the adenosine-receptor mediating the augmentation of histamine-induced inositol phospholipid hydrolysis in guinea-pig cerebral cortex

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1 Incubation (45 min) of slices of guinea-pig cerebral cortex with adenosine alone had no significant effect on the accumulation of [^{3}H]-inositol phosphates but enhanced the response to histamine H_1-receptor stimulation in a concentration-dependent manner.

2 The effect of adenosine on agonist-stimulated inositol phospholipid hydrolysis appeared to be selective for histamine H_1-receptor stimulation since it did not augment the phosphoinositide responses to carbachol, noradrenaline, 5-hydroxytryptamine or elevated KCl.

3 The accumulation of [^{3}H]-inositol phosphates induced by histamine increased linearly between 5 and 45 min incubation with agonist. However, following the simultaneous addition of histamine and adenosine, there was a marked delay in the appearance of the augmentation produced by adenosine.

4 The augmentation of [^{3}H]-inositol phosphate accumulation was mimicked by a number of adenosine analogues. The rank order of potency was; cyclopentyladenosine > **R**-phenylisopropyladenosine > 5'-N-ethylcarboxamidoadenosine > 2-chloroadenosine. This is consistent with the order expected for an adenosine A_1-receptor effect but the EC_{50} values were in the micro- rather than nanomolar range.

5 The response to 2-chloroadenosine was antagonized by the xanthine adenosine-antagonists, cyclopropyltheophylline, 8-phenyltheophylline, 3-isobutyl-1-methylxanthine and theophylline, and the non-xanthine alloxazine.

Introduction

Adenosine is an inhibitory modulator of both central and peripheral neurotransmission (Snyder, 1985). Two adenosine-receptor subtypes, A_1 and A_2, have been identified in mammalian brain from studies of ligand-binding and adenylate cyclase activity (Daly *et al.*, 1981;1986; Bruns *et al.*, 1980; Snyder, 1985). Adenosine can both inhibit and stimulate adenylate cyclase activity via A_1 and A_2 receptors respectively (Sattin & Rall, 1970; Van Calker *et al.*, 1979; Cooper *et al.*, 1980; Bazil & Minneman, 1986; Daly *et al.*, 1986). In guinea-pig cerebral cortical slices the A_2-mediated stimulation of cyclic AMP accumulation can be augmented by histamine H_1-receptor, α-adrenoceptor or 5-HT_2 receptor stimulation (Daly, 1977; Hill *et al.*, 1981; Daum *et al.*, 1982; Hollingsworth & Daly, 1985). These latter receptor systems do not appear to be directly coupled to the cyclic AMP- generating enzyme adenylate cyclase but are linked instead, via inositol phospholipid breakdown, to the production of two different second messengers, diacylglycerol and inositol trisphosphate (Brown *et al.*, 1984; Daum *et al.*, 1984; Kendall & Nahorski, 1985; Donaldson & Hill, 1986a,b), that activate protein kinase C and Ca^{2+} mobilization respectively (Berridge, 1984).

Very recently, it has been suggested that a reverse interaction can occur between adenosine and histamine on phosphoinositide hydrolysis in guinea-pig cerebral cortical slices (Hollingsworth *et al.*, 1986; Hill & Kendall, 1986). Thus, these authors have reported that adenosine and the adenosine analogue, 2-chloroadenosine, can augment inositol phospholipid hydrolysis elicited by histamine but not that produced by noradrenaline or carbachol. In the present study we have undertaken a quantitative evaluation of this interaction between adenosine and histamine on inositol phosphate accumulation in guinea-pig cerebral cortical slices.

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Methods

Accumulation of [^{3}H]-inositol phosphates

[^{3}H]-inositol phosphate accumulation was measured in guinea-pig cerebral cortical slices essentially as described previously (Brown *et al.*, 1984) except that acid extraction was used to improve the recovery of the more polar inositol phosphates (Donaldson & Hill, 1986a). Briefly, guinea-pig (Hartley strain, 200–400 g, either sex) cerebral cortical slices (350 × 350 μm) were cut with a McIlwain tissue chopper and preincubated for 60 min in Krebs Henseleit buffer gassed with O_2/CO_2 (95:5). Slices were allowed to settle under gravity and 50 μl aliquots of the gently packed slices were further incubated for 45 min in flat-bottomed insert vials containing Krebs buffer, 0.3 to 1.0 μCi [^{3}H]-inositol and 5 mM LiCl in a final volume of 300 μl. Where appropriate, antagonist drugs were added in 10 μl medium 20 min before the end of this incubation period. Agonists were then added in 10 μl medium and the incubation terminated 45 min later by adding 100 μl of ice-cold perchloric acid (10% w/v). The samples were neutralized with ice-cold KOH (approximately 0.75 ml of 0.15 M) and centrifuged to precipitate $KClO_4$ (1000 *g*, 5 min). Aliquots (0.75 ml) of the supernatant were diluted to 3 ml with 50 mM Tris buffer, pH 7, and added to columns containing Dowex resin (X8, 100–200 mesh) in the chloride form. [^{3}H]-inositol was removed with 20 ml H_2O and total [^{3}H]-inositol phosphates were eluted with 2.5 ml of 1 M HCl. Radioactivity was determined by scintillation counting in the gel phase (Emulsifer Scintillator 199, Packard). Parallel experiments, using Dowex resin converted to the formate form to separate inositol phosphates (Brown *et al.*, 1984; Donaldson & Hill, 1986a) gave very similar results to those obtained with Dowex resin in the chloride form. In some experiments, the formate resin was used to separate the individual mono-, bis-, tris- and tetrakis-phosphates according to Batty *et al.* (1985) and Donaldson & Hill (1986a).

Analysis of data

Concentration-response curves for agonist-stimulated [^{3}H]-inositol phosphate accumulation were fitted to a Hill equation using the programme ALLFIT (De Lean *et al.*, 1978) as described previously (Donaldson & Hill, 1985). The actual equation fitted was: Stimulation of [^{3}H]-inositol phosphate accumulation = E_{max} $D^n/(D^n + (EC_{50})^n)$ 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 test for differences in the histamine dose-response parameters, obtained in the presence and absence of adenosine, by inspecting the effect on the residual variance of forcing them to be equal (De Lean *et al.*, 1978; Donaldson & Hill, 1986b).

Affinity constants (K_a) for adenosine receptor antagonists were obtained from the parallel shift of the log dose-response curves to 2-chloroadenosine using the relationship:

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

where A is the concentration of antagonist and the dose-ratio is the ratio of the concentration of 2-chloroadenosine necessary to give a specified response in the presence of antagonist to the concentration of 2-chloroadenosine required for the same response in the absence of antagonist.

Chemicals

[^{3}H]-myo-inositol (16.5 Ci mmol^{-1}) was purchased from New England Nuclear. 2-Pyridylethylamine and 8-cyclopropyltheophylline were generous gifts from Smith, Kline & French Labs. and Abbot Labs. respectively. Alloxazine was obtained from Aldrich Chemical Company. All other chemicals and biochemicals were purchased from Sigma Chemical Company or Fisons Scientific Equipment.

Results

Effect of adenosine on inositol phosphate accumulation

Incubation (45 min) of slices of guinea-pig cerebral cortex with 0.1 mM adenosine alone had no significant effect on the accumulation of [^{3}H]-inositol phosphates but enhanced the response to histamine H_1-receptor stimulation in a concentration-dependent manner (Figure 1a). The extent of the augmentation produced by adenosine varied between experiments producing a 1.3 to 4.4 fold increase in the size of the maximum response to histamine over 29 experiments; mean = 2.3 ± 0.1 fold. The increased response to histamine was due to a significant increase in the maximum response ($P < 0.005$, analysis of variance according to De Lean *et al.*, 1978) and no consistent change was observed in the EC_{50} value (Figure 1b). The mean ratios of EC_{50} values (EC_{50} [+ adenosine]/EC_{50} [− adenosine]) obtained for histamine in the presence of 0.1 mM or 0.3 mM adenosine were 1.4 ± 0.3 and 0.6 ± 0.1 respectively. Following 45 min incubation with 0.1 mM histamine the proportion of tritium present in the individual inositol phosphate fractions represented 69.6, 19.6, 9.7 and 1.1% of the total inositol phosphates for the inositol mono-, bis-, tris- and tetrakis-phosphates respectively. In the presence of 0.1 mM adenosine there was no marked change in

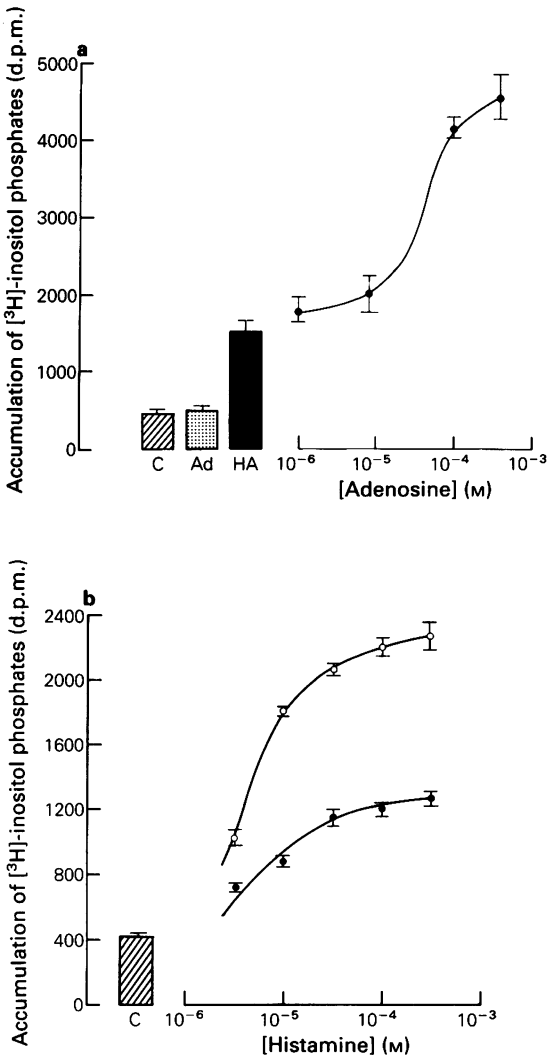

Figure 1 Concentration-response curves for (a) the augmentation by adenosine of the accumulation of [^{3}H]-inositol phosphates elicited by histamine (0.1 mM) (●) and (b) the stimulation by histamine of [^{3}H]-inositol phosphate accumulation in the presence (○) and absence (●) of 0.1 mM adenosine. The basal accumulation of [^{3}H]-inositol phosphates occurring under these conditions is indicated by the histogram marked C and the responses obtained with 0.1 mM histamine (HA) or 0.1 mM adenosine (Ad) alone are also shown as histographs. Results are expressed as d.p.m. in total [^{3}H]-inositol phosphates. The figures represent the results of single experiments. Each data point represents the mean of five (a) or four (b) replicate determinations; vertical lines show s.e.mean. Where appropriate, adenosine and histamine were added simultaneously. The experiments were repeated three times with essentially similar results.

the proportions of these major fractions (75.5, 15.5, 8.2 and 0.8% respectively).

The effect of adenosine on agonist-stimulated inositol phospholipid hydrolysis appeared to be selective for histamine H_1-receptor-mediated responses since it did not augment the phosphoinositide responses to carbachol (30 μM), noradrenaline (300 μM), 5-HT (300 μM) or KCl (31 mM) (Figure 2). Adenosine did, however, augment the inositol phospholipid response to the H_1-selective agonist 2-pyridylethylamine (Figure 3). This agent acts as a partial H_1-agonist in guinea-pig cerebral cortex (EC_{50} 26 μM; maximum response 35% of maximum response to histamine; Donaldson, 1986; Donaldson & Hill, 1986b) and this property of 2-pyridylethylamine was conserved in the presence of adenosine (0.1 mM). The extent of the stimulation produced by 2-pyridylethylamine was increased 3.5 ± 0.6 ($n = 3$) fold in the presence of adenosine, while the response to histamine was increased 2.6 ± 0.7 fold in the same experiments ($n = 3$). The response to a combination of adenosine and histamine was significantly antagonized by the selective H_1-receptor antagonist, mepyramine (Figure 3). This suggests that the increased response to histamine was mediated by H_1-receptors and not due to a non-H_1-effect of histamine on inositol phospholipid metabolism such as that observed in guinea-pig ileum (Donaldson & Hill, 1985; 1986a).

The accumulation of [^{3}H]-inositol phosphates induced by histamine increased linearly between 5 and 45 min incubation with agonist. Following the simultaneous addition of histamine and adenosine, there was a delay in the appearance of the augmentation produced by adenosine (Figure 4). A significant enhancement of the response to histamine was only observed after 20 min (Figure 4, 2 experiments) or 30 min (1 experiment) incubation with adenosine.

Adenosine-receptor agonists

The potentiation of the inositol phospholipid response to histamine produced by adenosine was also observed with a number of adenosine-receptor agonists including **R**-N^6-phenyl-isopropyl-adenosine (R-PIA), 2-chloroadenosine, cyclopentyladenosine and 5'-N-ethylcarboxamidoadenosine (NECA) (Figure 5). The dose-response curves to these agents were characteristically biphasic and a reduction in the maximal response was normally observed at higher concentrations (Figure 5). In Figure 5 the curve for 2-chloroadenosine appears to plateau at 87%. This reflects the fact that the responses are expressed as a percentage of the maximum response obtained in each individual experiment and that the 'peak' concentration for 2-chloroadenosine varied between experiments. The 'bell-shape' character of the concentration-response curve obtained for 2-chloroaden-

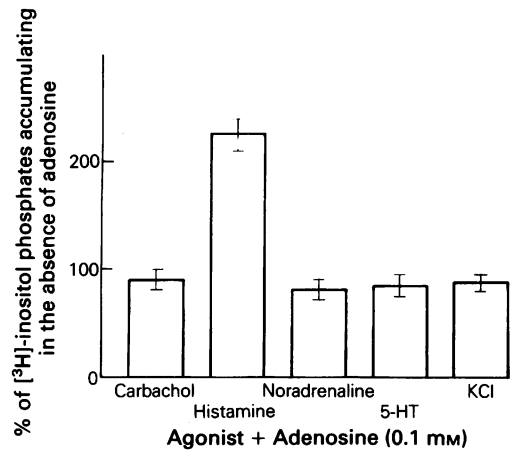

Figure 2 Influence of adenosine on the phosphoinositide responses to different stimulants. The ordinate scale shows the stimulation (minus basal) of [^{3}H]-inositol phosphate accumulation obtained with the different agonists in the presence of adenosine (0.1 mM) expressed as a percentage of the response obtained with the same agonists in the absence of adenosine. Values represent the mean of three separate experiments in which each incubation was conducted in quadruplicate; vertical lines show s.e.mean. The total d.p.m. in the [^{3}H]-inositol phosphate fraction after 45 min with agonist alone were 16,183 ± 1114 (30 μM carbachol), 1912 ± 156 (100 μM histamine), 4874 ± 540 (300 μM noradrenaline), 2839 ± 311 (300 μM 5-HT) and 9625 ± 670 (31 mM KCl). The control incubations contained 1008 ± 121 d.p.m. Where appropriate, agonist and adenosine were added simultaneously.

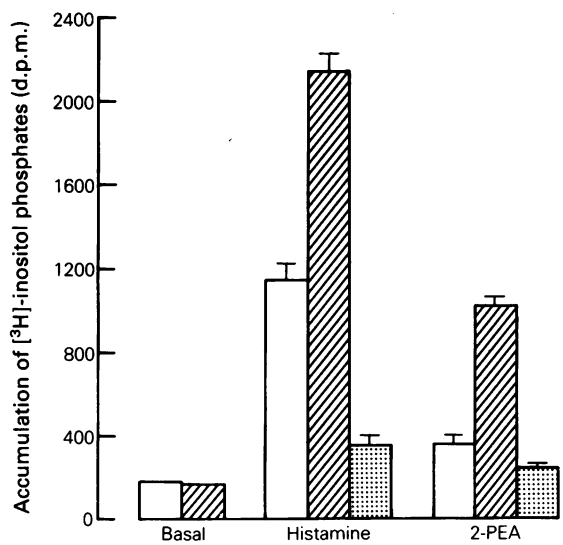

Figure 3 Effect of adenosine on the inositol phospholipid response to 2-pyridylethylamine in guinea-pig cerebral cortical slices. The open columns represent the accumulation of [^{3}H]-inositol phosphates occurring under basal conditions or in response to histamine (0.1 mM) and 2-pyridylethylamine (2-PEA, 1 mM). The remaining columns represent the responses obtained to these stimuli in the presence of adenosine (0.1 mM; hatched bars) or a combination of adenosine and mepyramine (1 μM; stippled bars). Where appropriate, agonist and adenosine were added simultaneously and mepyramine was added 20 min before agonist administration. Values represent mean of quadruplicate determinations in a single experiment; vertical lines show s.e.mean. Two other experiments gave very similar results.

osine in individual experiments is illustrated in Figure 7. The dose-response curves for adenosine analogues are rather steep and in order to obtain an estimate of the slope parameters the curves in Figure 5 were fitted (ALLFIT) to a Hill equation as described under Methods. To limit the extent to which the reduced response at high agonist concentrations interfered with the curve-fitting routine the data points obtained at 0.1 mM 2-chloroadenosine and 0.1 mM PIA were omitted from the analysis. It was notable that the Hill coefficients obtained for all of the adenosine analogues tested were close to 2.0. The values obtained for the EC_{50} of each adenosine agonist from this analysis are set out in Table 1.

The maximal extent of the augmentation of the response to histamine produced by the different adenosine analogues were similar to that produced by 3×10^{-4} M adenosine or 10^{-4} M adenosine in the presence of dipyridamole (0.5 μM) (Figure 6).

The dose-response curve for adenosine itself was not particularly well defined because of the high EC_{50} value which was *circa* 5×10^{-5}M. However, following blockade of adenosine transport with dipyridamole (0.5 μM) the EC_{50} for adenosine was reduced to 9.9 μM (Figure 5, Table 1) with no significant change in the maximum response.

Adenosine-receptor antagonists

The augmentation of the inositol phospholipid response to histamine (0.1 mM) produced by adenosine (0.1 mM) was completely antagonized by the adenosine receptor antagonists, theophylline (1 mM) and 3-isobutyl-1-methylxanthine (1BMX, 1 mM). These agents did not, however, alter the basal response to histamine alone. In order to obtain a quantitative assessment of the antagonist potencies of these agents, concentration-response curves to the more potent agonist 2-chloroadenosine were obtained in the presence and absence of a fixed concentration of each adenosine receptor antagonist (Figure 7). Each

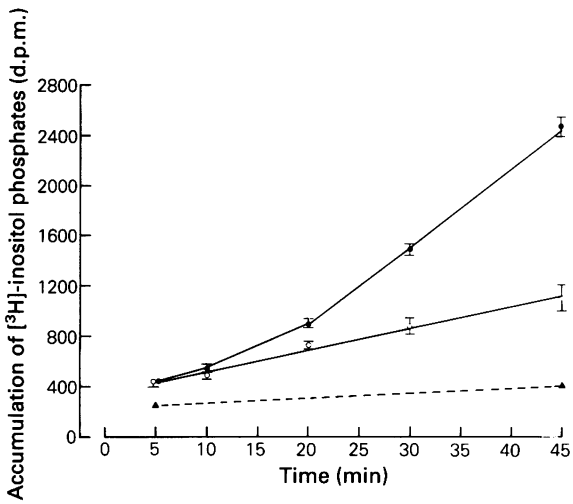

Figure 4 Time course of the stimulation of [^{3}H]-inositol phosphate accumulation elicited by histamine (1 mM) (○) or a combination of histamine (1 mM) and adenosine (0.3 mM) (●) in slices of guinea-pig cerebral cortex. (▲) Basal accumulation. Values represent mean of quadruplicate determinations in a single experiment; vertical lines show s.e.mean. Where appropriate, adenosine and histamine were added simultaneously. Two other experiments gave essentially similar results.

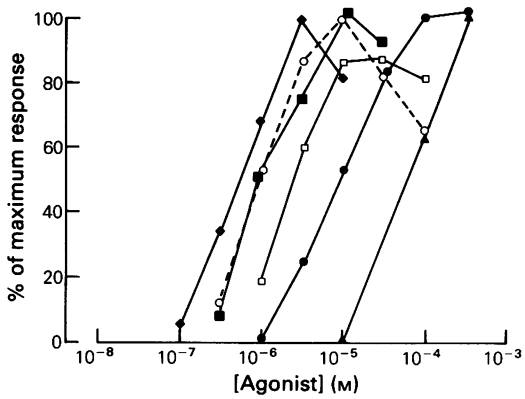

Figure 5 Augmentation of the histamine-stimulated accumulation of [^{3}H]-inositol phosphate accumulation in guinea-pig cerebral cortical slices by adenosine-receptor agonists. Incubations, containing 1 mM histamine were as described under Methods. Histamine and adenosine-receptor agonists were added simultaneously. To normalise responses from different slice preparations, responses are expressed as a percentage of the maximal response to the agonist obtained in each experiment. Each point represents the combined mean from 7 (2-chloroadenosine) or 3 (other agonists) separate experiments. (◆) Cyclopentyladenosine; (○) R-phenylisopropyladenosine (R-PIA); (■) 5'-N-ethylcarboxamidoadenosine (NECA); (□) 2-chloroadenosine; (●) adenosine + dipyridamole (0.5 μM); (▲) adenosine.

Table 1 EC_{50} values for adenosine-receptor agonist potentiation of histamine-induced inositol phospholipid hydrolysis in guinea-pig cerebral cortical slices

Agonist	EC_{50} (μM)	(n)
Cyclopentyladenosine	0.4 ± 0.1	(3)
PIA	0.8 ± 0.1	(3)
NECA	0.9 ± 0.1	(3)
2-Chloroadenosine	1.9 ± 0.2	(7)
Adenosine + dipyridamole (0.5 μM)	9.9 ± 1.5	(3)

Values for the EC_{50} were obtained from non-linear least squares analysis of the data in Figure 5 as described under Methods. To limit the extent to which the inhibition of [^{3}H]-inositol phosphate accumulation at high agonist concentrations interfered with the fitting procedure, the data obtained with 0.1 mM PIA and 0.1 mM 2-chloroadenosine were not included in this analysis. The numbers of individual agonist dose-response curves combined for each analysis are shown in parentheses. NECA: 5'-N-ethylcarboxamidoadenosine; PIA: phenylisopropyladenosine.

antagonist studied produced a parallel displacement of the concentration-response curve to 2-chloroadenosine, appearing to antagonize both aspects of the 'bell-shaped' dose-response curve (Figure 7). The extent of the blockade produced by each antagonist was quantified in terms of the parallel shift of the rising phase of the agonist curve and the apparent affinity constants determined in this way are set out in Table 2.

The lack of effect of adenosine-receptor antagonists on the response to histamine alone suggests that the response to this amine does not include a component due to any augmentation produced by endogenous adenosine. This finding was confirmed in studies using the adenosine metabolising enzyme adenosine deaminase (Figure 8). Studies with tetrodotoxin (5 μM) also suggest that the augmentation produced by exogenous adenosine is not mediated indirectly by release of another neuromodulator (Figure 8).

Discussion

It has been proposed that adenosine acts as a neuromodulator in the mammalian CNS (Snyder,

Table 2 Affinity constants obtained for adenosine-receptor antagonists from inhibition of 2-chloroadenosine-induced potentiation of inositol phospholipid hydrolysis

Antagonist	*Affinity constant (M^{-1}) from inhibition of:* *Inositol phosphate accumulation*	*Cyclic AMP accumulation**	*[^{3}H]-CHA binding***
Cyclopropyltheophylline	$2.2 \pm 0.6 \times 10^6$		
8-Phenyltheophylline	$9.7 \pm 0.6 \times 10^5$	5.5×10^6	1.2×10^7
IBMX	$4.5 \pm 2.7 \times 10^5$	2.9×10^5	4.0×10^5
Theophylline	$5.8 \pm 1.2 \times 10^4$	2.1×10^5	1.2×10^5
Alloxazine	$5.0 \pm 1.0 \times 10^4$	9.0×10^5	1.9×10^5

Values for inositol phosphate accumulation represent mean ± s.e.mean of three determinations. *Values found for inhibition of A_2-receptor mediated cyclic AMP accumulation in human fibroblasts (Bruns *et al.*, 1986). **Values obtained for inhibition of A_1-receptor binding of [^{3}H]-cyclohexyladenosine ([^{3}H]-CHA) (Bruns *et al.*, 1986). IBMX: 3-isobutyl-1-methylxanthine.

1985) and it appears to exert many of its actions by inhibiting neurotransmitter release (Phillis & Barraco, 1985). Adenosine can alter the intracellular levels of the second messenger cyclic AMP by either stimulating (via A_2-receptors) or inhibiting (via A_1-receptors) adenylate cyclase activity (Bruns *et al.*, 1980; Daly *et al.*, 1981; 1986; Snyder, 1985). The present study confirms and extends the earlier observations made by Hollingsworth *et al.* (1986) and ourselves (Hill & Kendall, 1986) that adenosine can also modulate the activity of the inositol phospholipid second messenger system.

The augmentation produced by adenosine and its

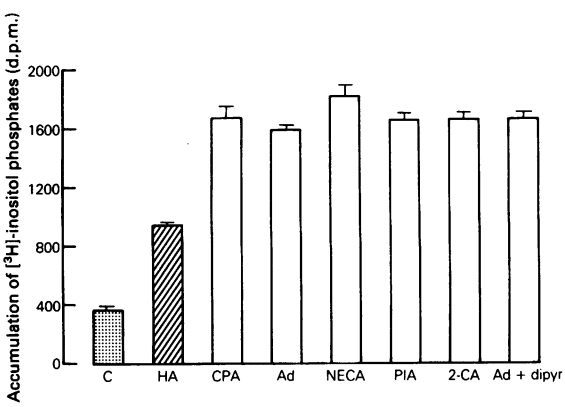

Figure 6 Comparison of the maximal [^{3}H]-inositol phosphate responses elicited by adenosine-receptor agonists. Results are expressed as d.p.m. in total [^{3}H]-inositol phosphates accumulating in response to cyclopentyladenosine (CPA, 3 μM); adenosine (Ad, 0.3 mM); 5'-N-ethylcarboxamidoadenosine (NECA, 10 μM); R-phenylisopropyladenosine (R-PIA, 10 μM); 2-chloroadenosine (2-CA, 30 μM) and adenosine (0.1 mM) + dipyridamole (0.5 μM) (Ad + dipyr) in the presence of histamine (1 mM). The basal and control response to histamine alone are indicated by the stippled and hatched columns respectively. Histamine and adenosine-receptor agonists were added simultaneously. Values represent mean of quadruplicate determinations in a single experiment; vertical lines show s.e.mean. A second experiment gave very similar results.

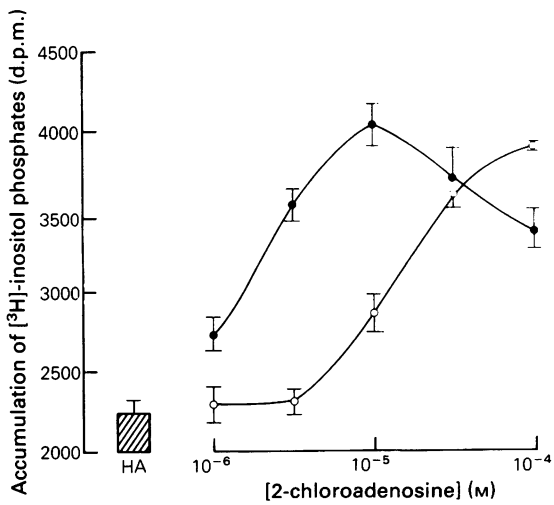

Figure 7 Inhibition by cyclopropyltheophylline of the potentiation by 2-chloroadenosine of histamine-induced accumulations of [^{3}H]-inositol phosphates in slices of guinea-pig cerebral cortex. Slices of guinea-pig cerebral cortex were incubated as described under Methods with increasing concentrations of 2-chloroadenosine in the presence (○) or absence (●) of cyclopropyltheophylline 5 μM. Histamine, 1 mM, was present throughout. Each point represents the mean of quadruplicate incubations from a single experiment; vertical lines show s.e.mean. The control histamine-stimulated level of [^{3}H]-inositol phosphate accumulation is shown by the hatched column.

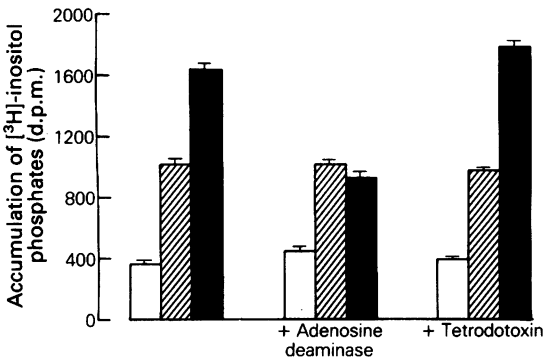

Figure 8 Influence of adenosine deaminase (1.2 u ml^{-1}) and tetrodotoxin (5 μM) on the inositol phospholipid responses to histamine and adenosine. Results represent the accumulation of [^{3}H]-inositol phosphates obtained in response to histamine (0.1 mM; hatched column) or a combination of histamine (0.1 mM) and adenosine (0.1 mM) (solid column). The basal accumulation is given by the open column. Values represent mean of five replicate determinations in a single experiment; vertical lines show s.e.mean. Adenosine deaminase and tetrodotoxin were added 20 min before the application of agonists.

analogues appears to be specific for histamine H_1-receptor-mediated inositol phospholipid hydrolysis and no effect was observed on the inositol phosphate responses to muscarinic, α-adrenoceptor and 5-HT-(probably 5-HT_2; Hollingsworth & Daly, 1985) receptor stimulation or potassium-induced depolarization. The augmentation produced by adenosine is primarily a consequence of an increased maximal response to histamine. A similar effect of adenosine can be demonstrated on the response to 2-pyridylethylamine, although the partial H_1-agonist nature of this compound (Daum *et al.*, 1982; Donaldson & Hill, 1986b) is conserved in the presence of the nucleoside.

Adenosine is known to produce a marked stimulation of cyclic AMP accumulation in guinea-pig cerebral cortical slices (Daly, 1977; and references therein). Furthermore, the presence of a synergistic interaction between adenosine and H_1-receptor stimulation on cyclic AMP accumulation in slices of this brain region is now well established (Daly, 1977; Hill *et al.*, 1981; Daum *et al.*, 1982; Hollingsworth & Daly, 1985). Thus, it might be argued that the effect of adenosine on the inositol phospholipid metabolic cycle is mediated via cyclic AMP. However, the lack of effect of adenosine on the inositol phospholipid responses to noradrenaline and 5-HT, which also produce a large accumulation of cyclic AMP in the presence of adenosine in this tissue (Schultz & Daly, 1973a,b), makes this explanation unlikely.

The ineffectiveness of adenosine-receptor antagonists on the response to histamine alone suggests that endogenous adenosine does not contribute to the histamine response to the same extent as it does when cyclic AMP accumulation is measured. In this latter case, a significant cyclic AMP response can be demonstrated in guinea-pig cerebral cortical slices resulting from an interaction between histamine and endogenous adenosine, which is abolished by adenosine deaminase treatment (Hill *et al.*, 1981). A similar treatment of slices with this adenosine metabolising enzyme was, however, without effect on the inositol phospholipid response to histamine (Figure 8).

The time course of the synergism between histamine and adenosine on inositol phosphate accumulation is rather unusual in that the augmentation produced by adenosine was delayed by 20–30 min, even though the two agonists were added simultaneously. One possible explanation of this latency is that it reflects the time required for adenosine to diffuse into the slice preparation and equilibrate with the receptor population. Latencies of this order have certainly been observed with the more potent lipid-soluble analogues of adenosine such as R-PIA in slices of rat hippocampus (Dunwiddie & Fredholm, 1985). However, the electrophysiological response to adenosine itself is very rapid in onset (Dunwiddie & Fredholm, 1985). Consequently, other possibilities including effects on protein synthesis and protein phosphorylation will need to be considered in future studies designed to investigate this delay.

The adenosine analogues, cyclopentyladenosine, R-PIA, NECA and 2-chloroadenosine, all have very similar potencies for the augmentation of histamine-induced inositol phosphate accumulation, while adenosine is a much weaker agonist even after inhibition of adenosine transport with dipyridamole. This structure-activity relationship differs somewhat from that reported by Hollingsworth *et al.* (1986) who found that R-PIA was approximately ten fold weaker (EC_{50} 7 μM) than in the present study. Nevertheless, the relatively high potencies of cyclopentyladenosine, 2-chloroadenosine and cyclohexyladenosine (with respect to NECA) found in the present study and that of Hollingsworth *et al.* (1986) suggest that the receptor involved is not that of the A_2-class that mediates the cyclic AMP response in guinea-pig cerebral cortical slices (Hollingsworth *et al.*, 1986). Furthermore, it is striking that the rank order of potencies; cyclopentyladenosine > R-PIA > NECA > 2-chloroadenosine is consistent with the order reported by Bruns *et al.* (1986) for A_1-receptor binding. The stereoselectivity of the effects of R- and S-PIA on inositol phospholipid metabolism (Hollingsworth *et al.*, 1986) also suggests an A_1-involvement. However, it is important to note that these analogues inhibit [^{3}H]-cyclohexyladenosine binding (Bruns *et al.*, 1986) in the nanomolar concentration range and yet stimulate the

inositol phospholipid response in the micromolar range. Caution is therefore needed in assigning this response to the A_1 category of adenosine-mediated effects, particularly in view of the low potency of adenosine itself.

A striking feature of the agonist concentration-response curves obtained in the present study is that they are characterized by a Hill slope close to 2.0. The only exception was the curve obtained for adenosine in the presence of dipyridamole. This suggests that there may be a positively cooperative interaction of agonists with the adenosine-receptor involved. An alternative, and perhaps more likely, explanation is that the opposing inhibitory response observed with high concentrations of adenosine analogues, but not adenosine itself, substantially limits the extent of the augmentation phase of the response. Under these conditions the Hill coefficient may well be overestimated. In this respect it is interesting that in mouse cerebral cortical slices only an inhibition of histamine-induced inositol phospholipid hydrolysis has been demonstrated in response to adenosine-receptor agonists (Hill & Kendall, 1987).

The response to 2-chloroadenosine in guinea-pig cerebral cortex was inhibited by four xanthine antagonists and the non-xanthine alloxazine. It was notable that both aspects of the 'bell-shaped' agonist-curves were inhibited by these antagonists suggesting that the stimulatory and inhibitory phases of the agonist curves are closely related. The affinity constants for these compounds, calculated assuming competitive antagonism, agreed well with those reported for typical A_1- and A_2- receptors, for which they are not selective. These data suggest that an extracellular receptor is involved in the augmentation response since the intracellular P-site (Daly *et al.*, 1981; Daly, 1982; Snyder, 1985) is not affected by xanthines. This suggestion is supported by the finding that the inositol phosphate response to adenosine was amplified and not reduced by dipyridamole, an inhibitor of adenosine transport in guinea-pig cerebral cortex (Barberis *et al.*, 1981). Furthermore, both NECA and R-PIA which are not substrates for the adenosine uptake system and are devoid of P-site activity (Daly, 1982) produce the same maximal response as adenosine and other analogues.

The level at which the interaction between adenosine and histamine occurs can only be the subject of speculation at the present time. A receptor-receptor interaction analogous to the benzodiazepine- GABA relationship (Costa & Guidotti, 1979) is possible. Alternatively, adenosine may act to inhibit the release of a transmitter such as glutamate which can inhibit inositol phospholipid metabolism (Baudry *et al.*, 1986). Although, it is notable that neither the response to histamine nor a combination of adenosine and histamine was modified by tetrodotoxin. A final possibility is that the synergism occurs at the post-receptor level and involves modifications of the inositol phospholipid metabolic cycle. However since adenosine did not significantly change the relative proportions of individual inositol phosphates it is unlikely that the activities of any of the inositol phosphate phosphatases were selectively altered. The latter measurements were made after 45 min stimulations and it is possible that very early events might be affected differently. It is also possible that the other branch of the inositol phospholipid signal transduction pathway, i.e. diacyglycerol production and subsequent protein kinase C activation could be a target for adenosine. These intriguing possibilities must be the subject of future studies.

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