Differences in the adenosine receptors modulating inositol phosphates and cyclic AMP accumulation in mammalian cerebral cortex

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1 2-Chloroadenosine stimulated adenosine 3':5'-cyclic monophosphate (cyclic AMP) accumulation and potentiated (guinea-pig) or inhibited (mouse) the histamine H₁-receptor-stimulated hydrolysis of inositol phospholipids in slices of guinea-pig and mouse cerebral cortex.

2 Two xanthine-based adenosine receptor antagonists were identified which were one order of magnitude more potent at the adenosine receptor mediating augmentation of the histamine-stimulated inositol phospholipid hydrolysis than at the receptor linked to cyclic AMP formation in guinea-pig cerebral cortical slices.

3 These compounds, 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) and 7-benzyl-3-(2-methylpropyl)xanthine (BMPX) retained their selectivity under near-identical incubation conditions in both assays.

4 These compounds also showed similar affinities and selectivity for the adenosine receptor mediating inhibition of histamine-stimulated inositol phospholipid hydrolysis in mouse cerebral cortical slices.

5 Inclusion of agents which give rise to, or mimic, high levels of cyclic AMP (forskolin (1 μ M), 8-bromo-cyclic AMP (1 mM) and vasoactive intestinal polypeptide (1 μ M)) in the incubation medium failed to mimic the action of 2-chloroadenosine on inositol phospholipid turnover in cerebral cortical slices from either species.

6 These data suggest that the adenosine receptor modulating the hydrolysis of inositol phospholipid in mouse and guinea-pig cerebral cortical slices is different from the adenosine receptor linked to cyclic AMP formation, and, furthermore, that the modulation of inositol phospholipid metabolism in either species is not mediated via alterations in cyclic AMP levels.

Introduction

It has been shown recently that adenosine can modulate histamine-induced inositol phospholipid hydrolysis in mammalian cerebral cortical slices (Hollingsworth *et al.*, 1986; Hill & Kendall, 1987; Kendall & Hill, 1988). This effect of adenosine on inositol phospholipid hydrolysis is manifested as an augmentation of the response to histamine H₁-receptor stimulation in guinea-pig cerebral cortex (Hollingsworth *et al.*, 1986; Hill & Kendall 1987), but as an inhibition of the H₁ response in mouse (Kendall & Hill, 1988) and human (Kendall & Firth, 1989) cerebral cortex. In all cases, the modulatory effect of adenosine appears to be confined to histamine H₁-receptor responses, since the inositol phospholipid hydrolysis induced by nor-adrenaline, carbachol, 5-hydroxytryptamine or KCl

is unchanged by adenosine receptor agonists (Hill & Kendall, 1987; Kendall & Hill, 1988). The identity of the adenosine receptor involved in these actions on inositol phospholipid is unclear. The rank order of agonist potencies is suggestive of the involvement of an A₁-type receptor in both guinea-pig and mouse brain (Hollingsworth *et al.*, 1986; Hill & Kendall, 1987; Kendall & Hill, 1988). However, the EC₅₀ values for these agonists are all in the micromolar range and lie within a very narrow band, i.e. the potencies of N⁶-cyclopentyladenosine and 5'-N-ethylcarboxamidoadenosine differ by only a factor of two in the guinea-pig, and between four and five in the mouse (Hill & Kendall, 1987; Kendall & Hill, 1988).

An interaction between adenosine and histamine on cyclic AMP accumulation is also seen in cerebral cortical slices from mouse and guinea-pig brain

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(Daly, 1977; Hill & Kendall, 1989). In this case, histamine H_1 -receptor stimulation (and also stimulation of certain other calcium-mobilising receptors) leads to a potentiation of the cyclic AMP accumulation induced by adenosine analogues through an adenosine A_2 -receptor (Daum *et al.*, 1982; Daly, 1977). This augmentation is presumably the result of some indirect action of H_1 -receptors since there is no direct effect of H_1 -receptor activation on cyclic AMP synthesis (Donaldson *et al.*, 1988b). A relationship between raised cyclic AMP levels and modulation of agonist-induced inositol phospholipid hydrolysis has been observed in a number of tissues, including airway smooth muscle (Hall *et al.*, 1989), platelets (Takai *et al.*, 1982; Watson *et al.*, 1984) and gastric mucosal cells (Puurunen *et al.*, 1987). This raises the possibility that the large accumulation of cellular cyclic AMP that occurs in the presence of both histamine and adenosine may mediate one or both of the actions of adenosine (augmentation or inhibition) on inositol phospholipid hydrolysis.

In the present study we have examined whether xanthine-based antagonists can be used to distinguish between the receptors responsible for the actions of adenosine on cyclic AMP and inositol phosphate accumulation in mammalian brain and whether there is a level of 'cross-talk' between the cyclic AMP and inositol phospholipid second messenger systems similar to that seen in peripheral tissues. A preliminary account of this work has been presented to the British Pharmacological Society (Alexander *et al.*, 1989).

Methods

[3H]-cyclic AMP accumulation

The formation of 3H -labelled cyclic AMP in [3H]-adenine-prelabelled slices of mouse or guinea-pig cerebral cortical slices was assayed essentially as described previously (Donaldson *et al.*, 1988a). Briefly, after 60 min equilibration at 37°C with Krebs Henseleit buffer gassed with $O_2:CO_2$ (95:5), cross-chopped slices of cerebral cortex ($350 \times 350 \mu m$) from mouse brain (black C57 strain; 25–40 g; either sex) or guinea-pig brain (Hartley strain; 200–600 g; either sex) were further incubated with 1.5 MBq of [3H]-adenine for 40 min. After washing three times with Krebs-Henseleit buffer, gravity-packed slices ($25 \mu l$ for mouse or $50 \mu l$ for guinea-pig) were then distributed into flat-bottomed vials containing antagonist where appropriate, and incubated for 20–30 min. Agonists were then added in $10 \mu l$ of medium to give a total volume of $300 \mu l$. The incubation was terminated after 10 min (unless otherwise specified)

with $200 \mu l$ of ice-cold 1 M HCl. [3H]-cyclic AMP was then isolated by sequential Dowex-alumina chromatography as described previously (Donaldson *et al.*, 1988b).

Accumulation of 3H -labelled inositol phosphates

The accumulation of 3H -labelled inositol phosphates was assayed essentially as previously described (Brown *et al.*, 1984). Briefly, cross-chopped slices were equilibrated for 60 min as described above and then distributed as aliquots ($25 \mu l$ for mouse or $50 \mu l$ for guinea-pig) into flat-bottomed vials for incorporation of [3H]-inositol (4–12 kBq) in Krebs-Henseleit buffer containing 5 mM LiCl for 40 min. Where appropriate, antagonist drugs were added during this incubation. Agonist drugs were then added in $10 \mu l$ of medium to give a final volume of $300 \mu l$, and the incubation was terminated after 45 min by the addition of $100 \mu l$ of ice-cold 10% (w/v) perchloric acid. After neutralisation with ice-cold 0.15 M KOH and centrifugation at $3000g$ for 10 min, $0.75 ml$ aliquots of the supernatant layer were diluted to 3 ml with 50 mM Tris, pH 7.0, and added to columns of Dowex 1 anion exchange resin (X8, 100–200 mesh, chloride form). [3H]-inositol was removed with 20 ml distilled water, and the total 3H -labelled inositol phosphates were eluted with 3 ml 1 M HCl. Radioactivity was quantified by scintillation counting in the gel phase (Opti Phase, LKB).

Data analysis

Antagonist equilibrium dissociation constants (K_d) were estimated by two methods. Firstly, concentration-response curves to 2-chloroadenosine were generated in the absence and presence of a fixed concentration of antagonist. The K_d was then calculated from the parallel log concentration-response curves from the relationship:

$$K_d = A/(DR - 1)$$

where A is the antagonist concentration, and DR represents the ratio of the concentrations of 2-chloroadenosine required to give a specified response in the presence and absence of antagonist.

Second, increasing concentrations of 2-chloroadenosine were used to generate dose-response curves, and a concentration (C) of 2-chloroadenosine was chosen which gave a response greater than 50% of the maximal agonist response. The concentration of antagonist (A_{50}) required to reduce the response elicited by this concentration of agonist (C) to 50% of the maximal response elicited in the absence of antagonist was then determined and the K_d was then

calculated from the relationship (Lazareno & Roberts, 1987):

$$K_d = A_{50}/(C/EC_{50} - 1)$$

Chemicals

Cimetidine and 8-cyclopropyl-1,3-dimethylxanthine (8-cyclopropyltheophylline) were generous gifts from Smith, Kline & French and Abbott Laboratories, respectively. 7-Benzyl-3-(2-methylpropyl)xanthine (BMPX) and 1,3-diethyl-8-phenylxanthine were synthesized by Dr R.J. Grout in the Department of Pharmaceutical Sciences, University of Nottingham. Adenosine deaminase ($183 \text{ } \mu\text{g}^{-1}$) and 1,3-dimethyl-8-phenylxanthine (8-phenyltheophylline) were purchased from Sigma. 8-Cyclopentyl-1,3-dipropylxanthine (DPCPX) was purchased from Research Biochemicals Incorporated. $[2\text{-}^3\text{H}]$ -inositol ($529 \text{ GBq mmol}^{-1}$) and $[8\text{-}^{14}\text{C}]$ -cyclic AMP ($1.6 \text{ GBq mmol}^{-1}$) were obtained from New England Nuclear. $[8\text{-}^3\text{H}]$ -adenine ($888 \text{ GBq mmol}^{-1}$) was obtained from Amersham International.

Results

Augmentation of inositol phosphates accumulation in guinea-pig cerebral cortex

Despite having no effect alone, 2-chloroadenosine potentiated the accumulation of $[^3\text{H}]$ -inositol phosphates induced by histamine (0.3 mM) over 40–45 min in guinea-pig cerebral cortex slices, as previously described (Hill & Kendall, 1987). The 2-chloroadenosine-mediated augmentation of the histamine response was antagonized by five xanthine-based compounds with K_d values varying from 10 nM to $5 \text{ } \mu\text{M}$ (Table 1). 8-Cyclopentyl-1,3-dipropylxanthine (DPCPX) was the most potent antagonist ($K_d = 0.010 \pm 0.002 \text{ } \mu\text{M}$), while the least potent compound

tested was 7-benzyl-3-(2-methylpropyl)xanthine (BMPX) with a K_d value of $5.0 \pm 1.6 \text{ } \mu\text{M}$ (Table 1). None of the five antagonists showed a significant alteration in the histamine-induced inositol phosphate response at the concentrations employed (e.g. Figure 3).

Stimulation of cyclic AMP formation in guinea-pig cerebral cortex

2-Chloroadenosine stimulated the formation of $[^3\text{H}]$ -cyclic AMP in guinea-pig cerebral cortical slices (10 min agonist incubation time) in a concentration-dependent manner, as previously described (Daly, 1977). DPCPX was the most potent of the antagonists studied with a K_d value ($0.082 \pm 0.008 \text{ } \mu\text{M}$, Table 1) which was significantly greater ($P < 0.05$, Student's *t* test) than that obtained from studies of inositol phosphate accumulation ($n = 3$, Table 1). Two of the other four compounds examined in this study had K_d values which were not significantly different for the two assay systems, however BMPX and 8-cyclopropyltheophylline were also able to discriminate between the two responses to 2-chloroadenosine (Table 1). At the concentrations used, all of the antagonists had little effect on basal cyclic AMP formation (e.g. see legend to Figure 2).

The determination of K_d values for the two most discriminatory compounds, DPCPX and BMPX, as antagonists for the adenosine receptor-mediated cyclic AMP and inositol phosphates responses were subsequently carried out under near-identical conditions. The agonist incubation period for the assay of $[^3\text{H}]$ -cyclic AMP was extended to 40–45 min to match that of the inositol phosphate assay. This led to no alteration in the concentration-response profile for 2-chloroadenosine (data not shown), in keeping with our previous observations that the steady state

Table 1 Antagonism of 2-chloroadenosine-mediated augmentation of inositol phosphate and cyclic AMP accumulation in guinea-pig cerebral cortical slices

Compound	K_d (μM)		Ratio (A/B)
	Cyclic AMP (A)	Inositol phosphates (B)	
DPCPX	0.082 ± 0.008	0.010 ± 0.002	8.2*
1,3-Diethyl-8-phenylxanthine	0.20 ± 0.03	0.60 ± 0.20	0.33
8-Phenyltheophylline	0.40 ± 0.20	0.80 ± 0.10	0.50
8-Cyclopropyltheophylline	2.3 ± 0.2	0.50 ± 0.10	4.6*
BMPX	40.0 ± 8.0	5.0 ± 1.6	8.0*

DPCPX = 8-cyclopentyl-1,3-dipropylxanthine; BMPX = 7-benzyl-3-(2-methylpropyl)xanthine.

Equilibrium dissociation constants (K_d) for xanthine derivatives for antagonism of 2-chloroadenosine-stimulated $[^3\text{H}]$ -cyclic AMP formation (agonist incubation time 10 min) and 2-chloroadenosine-potentiated histamine-induced $[^3\text{H}]$ -IP accumulation (agonist incubation time 40–45 min) in guinea-pig cerebral cortical slices. Values are mean $\pm$ s.e.mean of 3–4 separate experiments carried out with 3–4 replicates.

* $P < 0.05$; Student's *t* test; inositol phosphate K_d versus cyclic AMP K_d .

Figure 1 The effect of LiCl (5 mM) on 2-chloroadenosine-stimulated [^3H]-cyclic AMP accumulation in guinea-pig cerebral cortical slices. Agonist incubations were for 40–45 min in the presence of histamine (0.3 mM), cimetidine (0.3 mM) and adenosine deaminase (1 u ml^{-1}). Data represent mean of triplicate determinations obtained in the absence (○) and presence of LiCl (●) in a single experiment; vertical bars show s.e.mean. Where no error bars are shown, the error is less than the symbol size. Basal [^3H]-cyclic AMP formation was $864 \pm 79 \text{ d.p.m.}$ and $830 \pm 47 \text{ d.p.m.}$ in the absence and presence of LiCl, respectively.

levels of cyclic AMP stimulated by 2-chloroadenosine are well maintained in this tissue (Donaldson *et al.*, 1988b). Histamine (0.3 mM) and LiCl (5 mM) were also added to the incubation medium for the measurement of [^3H]-cyclic AMP. The incubations also contained cimetidine (0.3 mM) to preclude direct stimulation of [^3H]-cyclic AMP formation via histamine H_2 -receptors (Hegstrand *et al.*, 1976), and adenosine deaminase (1 u ml^{-1}) to negate any effects of endogenous adenosine.

In the presence of adenosine deaminase and cimetidine, histamine decreased the EC_{50} for 2-chloroadenosine from $45.0 \pm 4.2 \mu\text{M}$ ($n = 5$) to $22.6 \pm 2.2 \mu\text{M}$ ($n = 7$), while markedly increasing the maximal response of this compound from $15,660 \pm 75 \text{ d.p.m.}$ ($n = 4$) to $50,360 \pm 160 \text{ d.p.m.}$ ($n = 7$). This confirms the results of previous findings (Daum *et al.*, 1982; Hollingsworth & Daly, 1985; Hollingsworth *et al.*, 1986; Donaldson *et al.*, 1988b). The further inclusion of LiCl (5 mM) in the [^3H]-cyclic AMP accumulation assay led to no significant alteration in either the EC_{50} or the maximal response of 2-chloroadenosine (Figure 1). Under these near-identical incubation conditions, BMPX and DPCPX retained their ability to discriminate between the two responses to 2-chloroadenosine

Figure 2 Antagonism by 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) of 2-chloroadenosine-stimulated [^3H]-cyclic AMP formation in guinea-pig cerebral cortical slices. Incubations were for 40 min in the presence of histamine (0.3 mM), cimetidine (0.3 mM), adenosine deaminase (1 u ml^{-1}), and LiCl (5 mM). Measurements were made in the absence (○) and presence of 500 nM DPCPX (●). Data represent mean of triplicate determinations in a single experiment; vertical bars show s.e.mean. Similar results were obtained in two further experiments. Basal [^3H]-cyclic AMP formation was 457 ± 5 and $492 \pm 4 \text{ d.p.m.}$ in the absence and presence of DPCPX, respectively.

(Table 2). Thus, as illustrated in Figure 2, 500 nM DPCPX induced a small rightward shift in the 2-chloroadenosine stimulatory response for cyclic AMP levels, while a lower concentration (100 nM) produced a much larger shift in the 2-chloroadenosine concentration-response curve for augmentation of the histamine H_1 -receptor-mediated inositol phosphates response (Figure 3). In the case of DPCPX, which proved to be approximately ten fold more potent as an antagonist of the adenosine receptor potentiating inositol phospholipid hydrolysis, K_d values were calculated by both of the methods described under Methods with similar results (Table 2).

Mouse cerebral cortical slices

In mouse cerebral cortical slices, the maximal response to 2-chloroadenosine with regard to cyclic AMP formation was low and often poorly defined. In the mouse, as in the guinea-pig, histamine was found to potentiate the maximal response of cyclic AMP, and to give better defined concentration-response curves. For example, in one experiment, the response to 0.3 mM 2-chloroadenosine was increased from $2802 \pm 271 \text{ d.p.m.}$ to $7692 \pm 499 \text{ d.p.m.}$ by the addition of 0.3 mM histamine (similar data were

Figure 3 Antagonism by 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) of the 2-chloroadenosine augmentation of histamine-induced $[^3\text{H}]$ -inositol phosphates accumulation in guinea-pig cerebral cortical slices. Incubation times were 45 min in the presence of cimetidine (0.3 mM), adenosine deaminase ($1 \mu\text{M}$) and LiCl (5 mM). Data represent means of triplicate determinations in a single experiment; vertical bars show s.e.mean. Similar data were obtained from two further experiments. (a) Basal (B) and histamine-stimulated (0.3 mM) $[^3\text{H}]$ -inositol phosphate accumulation in the absence (Hist) and presence of 100 nM DPCPX (Hist + DPCPX) are shown by the columns. Concentration-response curves to 2-chloroadenosine (in the constant presence of 0.3 mM histamine) were obtained in the absence (○) and presence of 100 nM DPCPX (●). (b) Inhibition of the augmented accumulation of $[^3\text{H}]$ -inositol phosphates induced by $30 \mu\text{M}$ 2-chloroadenosine (○) by increasing concentrations of DPCPX (●). Histamine (0.3 mM) was present in all incubations.

obtained in two other experiments). Consequently, measurements of antagonist K_d values were only performed in the presence of H_1 -receptor stimulation (Table 2).

In mouse cerebral cortical slices adenosine analogues inhibit histamine-stimulated inositol phospholipid hydrolysis (Figure 4; Kendall & Hill, 1988). However, the K_d values obtained for BMPX and DPCPX were similar to those obtained from antagonism of the potentiation of histamine-induced inositol phospholipid hydrolysis in guinea-pig brain (Table 2). Furthermore, BMPX and DPCPX were respectively 19 and 10 fold more potent at the receptor mediating inhibition of inositol phospholipid metabolism in the mouse than at the receptor involved in the stimulation of cyclic AMP levels (Table 2).

Effect of cyclic AMP stimulants

Agents that stimulate an increase in cyclic AMP levels (vasoactive intestinal polypeptide and forskolin), or that simulate high cyclic AMP levels (8-bromo-cyclic AMP) were unable to mimic the effects of adenosine analogues on histamine-stimulated inositol phospholipid hydrolysis in either guinea-pig or mouse cerebral cortex (Figure 5).

Discussion

Initial experiments using guinea-pig cerebral cortical slices identified two adenosine receptor antagonists, DPCPX and BMPX, which appeared to distinguish between two second messenger responses to 2-

Table 2 Antagonism of 2-chloroadenosine-mediated stimulation of cyclic AMP and augmentation or inhibition of inositol phosphates accumulation in guinea-pig and mouse cerebral cortical slices

	K_d (μM)		
	Cyclic AMP (- histamine)	Cyclic AMP (+ histamine)	Inositol phosphates (+ histamine)
<i>Guinea-pig</i>			
DPCPX ¹	0.073 ± 0.006 (3)	0.131 ± 0.026 (4)	$0.012 \pm 0.004^*$ (3)
DPCPX ²	NA	0.149 ± 0.036 (3)	$0.008 \pm 0.002^*$ (4)
BMPX ¹	77.0 ± 6.3 (3)	53.0 ± 1.0 (3)	$10.7 \pm 0.7^*$ (3)
<i>Mouse</i>			
DPCPX ¹	NA	0.113 ± 0.013 (5)	$0.010 \pm 0.003^*$ (4)
DPCPX ²	NA	0.220 ± 0.052 (3)	$0.024 \pm 0.004^*$ (5)
BMPX ¹	NA	150.0 ± 21.0 (3)	$8.0 \pm 3.2^*$ (3)

DPCPX = 8-cyclopentyl-1,3-dipropylxanthine; BMPX = 7-benzyl-3-(2-methylpropyl)xanthine.

Equilibrium dissociation constants (K_d) were determined in the presence or absence of 0.3 mM histamine. 5 mM LiCl, 0.3 mM cimetidine and $1 \mu\text{M}$ adenosine deaminase were present in all incubations. Agonist incubation times were 40–45 min throughout. Values are means $\pm$ s.e.mean of triplicate determinations from the number of separate experiments indicated in parentheses. K_d values were determined using either a fixed antagonist concentration¹ or by the method of Lazareno & Roberts (1987)² as described under *Methods*. NA = not available.

* $P < 0.05$; Student's t test inositol phosphates K_d versus cyclic AMP K_d in the presence of histamine.

Figure 4 Antagonism by 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) of the 2-chloroadenosine inhibition of histamine-induced [³H]-inositol phosphates accumulation in mouse cerebral cortical slices. Incubations were for 45 min and contained cimetidine (0.3 mM), adenosine deaminase (1 μM), and LiCl (5 mM). Data represent mean of triplicate determinations in a single experiment; vertical bars show s.e.mean. Similar data were obtained in two further experiments. Basal (B), histamine-stimulated (0.3 mM) (Hist) and histamine-stimulated [³H]-inositol phosphates accumulation in the presence of 100 nM DPCPX (Hist + DPCPX) are shown by the columns. Concentration-response curves to 2-chloroadenosine (in the constant presence of 0.3 mM histamine) were obtained in the absence (○) and presence of 100 nM DPCPX (●).

Figure 5 The effect of cyclic AMP stimulants or a cyclic AMP analogue on [³H]-inositol phosphates accumulation in (a) guinea-pig or (b) mouse cerebral cortical slices. Forskolin (For, 1 μM), 8-bromo-cyclic AMP (8Br, 1 mM) and vasoactive intestinal polypeptide (VIP, 1 μM) were present during the determination of triplicate determinations of basal (open bars) and histamine-stimulated (0.3 mM, shaded bars) [³H]-inositol phosphates accumulation. Values represent means $\pm$ s.e.mean from three separate experiments conducted in quadruplicate.

chloroadenosine. That is, they were approximately 10 fold more potent as antagonists of the receptor augmenting histamine H_1 -receptor-induced inositol phospholipid turnover compared with their potencies as antagonists of the receptor which stimulates cyclic AMP accumulation. However, the standard protocols for the two assays differ in certain aspects: the [³H]-cyclic AMP assay is carried out in the absence of lithium ions for 10 min, whereas the [³H]-inositol phosphate assay includes histamine and lithium in the incubation medium, with agonist incubation times of 45 min. Since it could be argued that the apparent differences in antagonist affinities are artifacts resulting from these procedural differences, the assays were standardized to common conditions.

Histamine is able to increase the formation of cyclic AMP in guinea-pig brain slices by both direct, via H_2 -receptors (Hegstrand *et al.*, 1976; Palacios *et al.*, 1978), and indirect, via H_1 -receptors, mechanisms (Daum *et al.*, 1982; Donaldson *et al.*, 1988b; Hill & Kendall, 1989). Consequently, cimetidine, a selective H_2 -receptor antagonist (Green *et al.*, 1977), was included in the assays to prevent the direct H_2 -receptor-mediated stimulation of cyclic AMP formation. Adenosine deaminase was also included to preclude any effects of endogenous adenosine. Neither cimetidine nor adenosine deaminase altered the affinities of DPCPX or BMPX for the adenosine receptor which augments the H_1 -receptor inositol phospholipid response (Tables 1 and 2). In the assay for cyclic AMP, histamine H_1 -receptor stimulation increased the apparent efficacy of 2-chloroadenosine as expected (Daly, 1977; Hollingsworth & Daly, 1985; Donaldson *et al.*, 1988b), and also decreased the EC_{50} for the agonist, although we are as yet unable to explain this latter effect.

The ability of DPCPX and BMPX to discriminate between the two adenosine receptor-mediated responses was retained under virtually identical assay conditions in guinea-pig cerebral cortical slices (Table 2). Similarly, in mouse cerebral cortical slices, DPCPX and BMPX were between 9 and 19 fold more potent at the adenosine receptor modulating inositol phospholipid turnover than at the cyclic AMP-stimulating receptor. From this it seems reasonable to conclude that the adenosine receptor which modulates the histamine H_1 -receptor inositol phospholipid response is different from the A_2 -receptor positively linked to cyclic AMP formation in both species. It also seems possible that the adenosine receptors augmenting inositol phospholipid metabolism in guinea-pig and inhibiting the same histamine response in mouse are very similar, if not identical.

The data obtained in this study further suggest that cyclic AMP does not mediate the effects of 2-

chloroadenosine on inositol phospholipid hydrolysis in cerebral cortical slices. The literature suggests some species or tissue differences in the effects of elevated cyclic AMP levels on inositol phospholipid metabolism. In platelets, lymphocytes and neutrophils, cyclic AMP appears to decrease receptor-induced inositol phospholipid hydrolysis (Kaibuchi *et al.*, 1982; Watson *et al.*, 1984; Della Bianca *et al.*, 1986; Jakobs *et al.*, 1986). In contrast, in rat vas deferens, in which adenosine augments α_1 -adrenoceptor-induced inositol phospholipid hydrolysis (Häggblad & Fredholm, 1987), the elevation of cyclic AMP levels or the use of cyclic AMP analogues failed to alter phenylephrine-stimulated inositol phospholipid breakdown. Our results using forskolin, vasoactive intestinal polypeptide, and 8-bromo-cyclic AMP indicate that accumulation of [3 H]-inositol phosphates in both guinea-pig and mouse cerebral cortical slices is unaffected by either raised cyclic AMP levels or the presence of cell-permeant cyclic AMP analogues. This is in accord with the results of Hollingsworth & Daly (1985) who reported no effect of forskolin on basal inositol phospholipid hydrolysis, or on that induced by noradrenaline, carbachol or histamine plus 2-chloroadenosine in guinea-pig cerebral cortical slices.

In this study, we have been able to distinguish by pharmacological means the adenosine receptor which modulates histamine-stimulated inositol phospholipid hydrolysis from the A_2 -receptor which stimulates cyclic AMP accumulation. We have previously been able to show that the intracellular P-site is not involved (Hill & Kendall, 1987; Kendall & Hill, 1988), since methylxanthines are ineffective at this latter adenosine 'receptor'. The rank order of agonist potencies for the receptor modulating inositol phospholipid turnover is similar to that for the A_1 -receptor, but, as previously noted, the absolute

potencies of agonists are considerably lower than those published for the A_1 -receptor in other systems (Hill & Kendall, 1987). Since DPCPX shows a greater than 700 fold selectivity for the A_1 -receptor compared to the A_2 -receptor (in both radioligand binding and adenylate cyclase studies; Bruns *et al.*, 1987; Lohse *et al.*, 1987) it was hoped that this compound would be useful in the identification of the inositol phospholipid-modulating adenosine receptor. However, the affinities of DPCPX at this receptor (K_d 8–24 nM, Tables 1 and 2) are inconsistent with the involvement of either A_1 - ($K_d \leq 0.5$ nM) or A_2 - (K_d 330–340 nM) receptors (Bruns *et al.*, 1987; Lohse *et al.*, 1987), although it is possible that these differences arise from differences in the tissue preparations employed, since the hydrophobic nature of the antagonists used may well lead to sequestration in structures not found in the disrupted cell.

In summary, the adenosine receptor responsible for the positive modulation of histamine-stimulated inositol phospholipid breakdown in guinea-pig cerebral cortical slices cannot be distinguished from that which inhibits the same response in mouse brain. This receptor is, however, different from the adenosine receptor which mediates a stimulation in cyclic AMP formation; a contention supported by the lack of effect of elevated levels of cyclic AMP or cyclic AMP analogues on the inositol phospholipid response in either species. It seems likely that the advent of more selective adenosine receptor antagonists or the application of molecular biological techniques will be necessary to classify positively the adenosine receptor which modulates CNS inositol phospholipid metabolism.

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