Biochemical Pharmacology, Vol. 35, No. 11, pp. 1809–1816, 1986.
Printed in Great Britain.

0006-2952/86 $3.00 + 0.00

INHIBITION OF IMMUNOLOGICAL AND NON-IMMUNOLOGICAL HISTAMINE RELEASE FROM HUMAN BASOPHILS BY ADENOSINE ANALOGUES THAT ACT AT P-SITES

PHILIP J. HUGHES and MARTIN K. CHURCH
Clinical Pharmacology, Centre Block, Southampton General Hospital, Southampton SO9 4XY, U.K.

(*Received* 10 *October* 1985; *accepted* 6 *December* 1985)

Abstract—2′,5′-Dideoxyadenoside (DDA) inhibited both anti-IgE and ionophore A23187 induced histamine secretion from human basophils. Whereas DDA inhibited IgE-dependent histamine secretion when added at all times prior to challenge, release induced by A23187 was inhibited only with simultaneous addition of DDA and secretagogue. Dipyridamole, but not theophylline, abrogated DDA mediated inhibition of histamine release suggesting an intracellular mechanism of action of DDA. The observations that 2′-deoxyadenosine and 9-β-D-arabinofuranosyladenine also inhibited release suggest that the inhibitory effect is produced by an interaction with internal P-sites. Furthermore, the observations that its inhibitory effect was enhanced by manganese and reversed by islet activating protein from *Bordetella pertussis* suggest that DDA inhibits basophil histamine release by interacting with a guanine nucleotide binding protein which may be linked to either adenylate cyclase or other second messenger system(s).

The role of cyclic AMP in activation-secretion coupling in mast cells and basophils is complex and poorly understood. A transient monophasic rise in cyclic AMP, which precedes calcium influxes and mediator release, is characteristic of immunologic activation of both cell types [1]. Adenosine analogues which interact with cell surface purinoceptors inhibit IgE-dependent histamine release when preincubated with human mast cells and basophils [2–5], but enhance release when added after challenge [3, 5]. In rodent mast cells only potentiation of histamine release occurs [6, 7]. All of these effects have been suggested to result from stimulation of adenylate cyclase activity following interaction of adenosine analogues with A_2-purinoceptors [7]. However, we have recently demonstrated that adenosine-induced enhancement of mediator release from rat mast cells is mediated by an atypical purinoceptor and is independent of cyclic AMP accumulation [8].

At high concentrations, adenosine and ribose modified analogues are taken into cells where they interact with P-sites on the internal surface of the cell membrane [9]. P-site activation is associated with an inhibition of adenylate cyclase activity [9]. Research into P-site effects has centred on 2′,5′-dideoxyadenosine (DDA), an adenosine analogue which has been chemically modified so that it neither interacts with cell surface purinoceptors nor couples with homocysteine to cause intracellular effects. DDA has been shown to inhibit IgE-dependent histamine release from preparations of rodent mast cells [7], human mast cells [2, 3] and human basophils [5]. Against non-immunologically induced histamine release, results are conflicting, some reports showing inhibition [10–12] whilst others show no effect [13]. Doubts that the effects of DDA are mediated by P-site stimulation have been raised by reports that two other P-site agonists, 9-β-D-arabinofuranosyladenine (ARA-A) and 2′-deoxyadenosine (2DA) do not inhibit immunological histamine release [10, 11].

This study demonstrated that DDA, ARA-A and 2DA inhibit both immunological and A23187-induced histamine release from human basophil leucocytes, but the characteristics of inhibition are different with the two stimuli. Theophylline and dipyridamole have been used to demonstrate that the effects of DDA are produced at an intracellular site. The observations that islet activating protein from *Bordetella pertussis* and manganese ions modulate DDA-mediated inhibition of histamine secretion whereas nicotinic acid mimics its effects suggest that DDA could be acting at the level of guanine nucleotide binding proteins which lead to inhibition of adenylate cyclase or other second messenger system(s).

MATERIALS AND METHODS

Materials. 2′,5′-Dideoxyadenosine (DDA) was supplied by P. L. Biochemicals (Milton Keynes, U.K.) and 2′-deoxyadenosine (2DA), 9-β-D-arabinofuranosyladenine (ARA-A), homocysteine thiolactone and nicotinic acid by Sigma (Poole, U.K.). Dipyridamole was purchased from C. H. Boehringer Sohn (Ingelheim Rhein, Germany) and purified islet activating protein from *Bordetella pertussis* from Dr L. Irons (PHLS, Porton Down, Salisbury, U.K.). Heat inactivated goat anti-human IgE serum was prepared as described previously [14]. Calcium ionophore A23187 (obtained from Sigma, Poole, U.K.) was diluted from a stock 1 mM solution in dimethylsulphoxide. All other reagents were of analytical grade. HEPES-buffered salt solution (HBSS) con-

tained 137 mM NaCl, 5.6 mM glucose, 10 mM *N*-2-hydroxyethyl-piperazine-*N'*-2-ethanesulphonic acid (HEPES), 0.5 mM NaH_2PO_4, 2.7 mM KCl and 0.03% human serum albumin, as was supplemented with 0.5 mM $MgCl_2$ and 1.0 mM $CaCl_2$. In experiments to assess the effects of manganese, magnesium was replaced with 1 mM $MnCl_2$. The pH of HBSS was adjusted to 7.4 by addition of aqueous NaOH immediately prior to use. Dextran used for leucocyte sedimentation was Dextraven 110 (Fisons, Loughborough, U.K.).

Separation of human basophil leucocytes. Fresh venous blood was anti-coagulated with 0.5 M ethylenediamine tetraacetic acid (1 vol. to 20 vol. blood) and the leucocytes separated by dextran sedimentation [15]. After washing twice in calcium and magnesium free HBSS the leucocytes were suspended in HBSS supplemented with 1 mM calcium and 0.5 mM magnesium.

Drug incubation and assessment of histamine release. Duplicate 200 μl aliquots of the leucocyte preparations containing $1–5 \times 10^5$ nucleated cells were incubated with 25-μl HBSS or drug under test for various periods of time before stimulation, with either 25 μl of anti-IgE (1/1000 final dilution) or ionophore A23187 (1 μM final concentration). After thorough mixing the histamine release reaction was allowed to proceed for 45 min at 37° before being stopped by centrifugation at 250 *g* for 10 min at 4°. The supernatant was removed, acidified with 250 μl of 10% trichloroacetic acid (TCA) and kept at −20° until assay. Total cellular histamine was measured in parallel tubes by disintegrating cells in 5% TCA. Histamine was measured by automated spectrofluorimetry [16]. Histamine release induced by secretagogues is expressed as a percentage of the total histamine release and corrected for spontaneous histamine release in the absence of secretagogue.

The effect of DDA on basal levels and anti-IgE induced changes in cellular cyclic AMP content were assessed using leucocytes separated from venous blood of a patient with a 40–70% basophilia [17]. Fifty microlitres of 4.5 mM DDA was added to 400 μl aliquots of leucocyte preparations containing 3×10^6 nucleated cells and incubated for various periods of time before challenge with 50 μl of 1/100 dilution anti-IgE (final dilution 1/1000). At various times after challenge the reactions were terminated by addition of 0.5 ml ice-cold ethanol whilst vortex mixing. The disrupted cell debris was precipitated by centrifugation at 400 *g* for 10 min at 4° and the separated supernatant was stored at −20° until assay. The cyclic AMP content of duplicate 100 μl aliquots of the supernatants was assayed by radioimmunoassay of acetylated samples [17]. Histamine release from duplicate aliquots of the same cells was assessed as described above.

Data analysis. The significance of the differences between release of histamine in the presence of the drug to that in its absence were tested by the Student's *t*-test for paired data. Concentration–response curves were constructed by least squares linear regression and compared by covariant analysis. Drug activities were expressed as the geometric mean concentrations of drug calculated to inhibit histamine secretion by 25% (IC_{25}). Net histamine release and inhibition of release are expressed as mean ± S.E.M.

RESULTS

Effect of time of addition of DDA with respect to challenge

The effect of a constant 500 μM concentration of DDA added to leucocyte preparations at time ranging from 15 min before to 15 min after challenge with anti-IgE (1/1000 dilution) was assessed in three experiments (Fig. 1). Added 15 min before challenge, DDA inhibited histamine release by 76.4 ± 6.2% ($P < 0.02$). As the preincubation time was shortened the degree of inhibition decreased. With simultaneous addition of DDA and anti-IgE, inhibition was 39.2 ± 0.9% ($P < 0.001$). Addition of DDA after challenge, when histamine release was already progressing also resulted in inhibition of release. When added 15 min after challenge, inhibition was 18.1 ± 0.2% ($P < 0.05$).

Histamine release induced by calcium ionophore A23187 (1 μM) was also inhibited by 500 μM DDA, but the pattern of inhibition was different to that observed with anti-IgE (Fig. 1). In three experiments, addition of DDA at times up to 2 min before challenge did not inhibit histamine release. When added simultaneously with A23187, DDA inhibited release by 27.4 ± 3.6% ($P < 0.02$). The inhibitory effects decreased rapidly when DDA was added after A23187 challenge, additions at 10 and 15 min after A23187 producing no significant effects.

Concentration-related effects of DDA

DDA, 50–1000 μM, produced concentration-related inhibitions of both anti-IgE and A23187-induced histamine release from human basophils

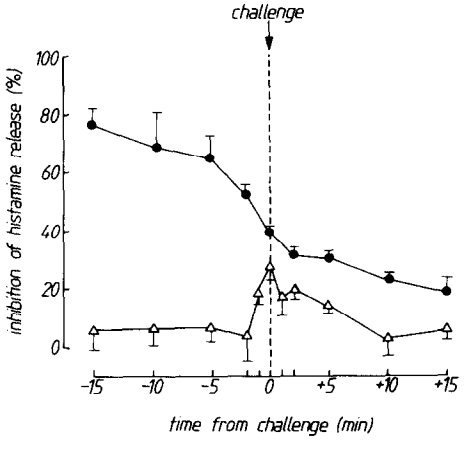

Fig. 1. The effect of the time of addition of DDA (500 μM) on histamine release from human basophil leucocytes produced by challenge with anti-IgE (●) or calcium ionophore A23187 (△). Each result is expressed as the mean percentage inhibition ±S.E.M. of three experiments where DDA was added at the specified times with respect to challenge with anti-IgE (1/1000 dilution: net histamine release was 30.9 ± 2.3%, spontaneous release was 7.8 ± 2.8%) or ionophore A23187 (1 μM: net histamine release was 58.5 ± 7.7%, spontaneous release was 6.0 ± 1.1%).

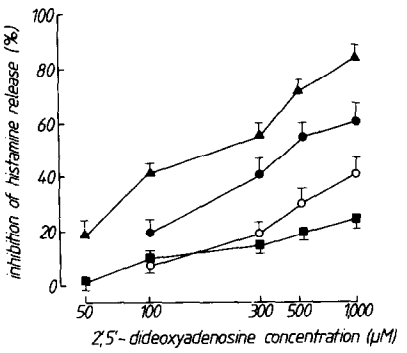

Fig. 2. Concentration related effects of DDA against histamine release from human basophil leucocytes when added 15 min before (▲), simultaneously with (●), 15 min after (■) anti-IgE challenge or simultaneously with (○) A23187 challenge. Each result for anti-IgE is expressed as the mean inhibition ±S.E.M. of 9 experiments in which the net histamine release was 34.6 ± 2.9% and spontaneous release was 6.6 ± 0.9%. Each result for A23187 is expressed as the mean inhibition ±S.E.M. in 19 experiments in which the net histamine release was 53.7 ± 5.1% and the spontaneous release was 6.8 ± 1.1%.

(Fig. 2). In nine experiments with anti-IgE as the secretory stimulus, DDA added 15 min before challenge caused a maximum of 84.2 ± 2.0% inhibition. The geometric mean concentration of DDA calculated to reduce histamine release by 25% (IC_{25}) was 46.7 μM (range 26.7–77.4 μM). When added simultaneously with anti-IgE, maximum inhibition was 60.8 ± 6.3% and the IC_{25} 234 μM (range 25.8–851 μM). When added 15 min after challenge, 1000 μM DDA produced 25.2 ± 1.5% inhibition of release. No IC_{25} was calculated. In 19 experiments in which DDA was added simultaneously with A23187 (Fig. 2), maximum inhibition of release was 41.1 ± 3.3% and the IC_{25} 415 μM (range 2.4–1659 μM).

Analysis of individual experiments showed that in all cases the concentration of DDA required to inhibit histamine release was directly related to the degree of histamine release in untreated cells. In experiments where DDA was added simultaneously with anti-IgE or A23187 challenge, the correlation coefficients for IC_{25} against histamine release in untreated controls were 0.82 and 0.79 (both $P < 0.01$) respectively. Comparison of these lines showed DDA to be approximately twice as effective at inhibiting anti-IgE induced histamine release when compared with that induced by A23187. The calculated IC_{25} values at matched histamine release were 270 μM and 450 μM respectively.

Effect of DDA on the time-course of histamine release

The effect of 1000 μM DDA on the time-course of histamine release induced by anti-IgE and A23187 was assessed in three experiments (Fig. 3). With both stimuli, the release of histamine was more rapid during the first 15 min than in the ensuing 30 min. At 15 min the extent of histamine release was 70–73% maximum. With addition of DDA at 15 min before anti-IgE challenge and simultaneously with anti-IgE or A23187 challenge, histamine release was significantly depressed at all time points, but the overall shape of the curves was unaltered. WHen DDA was added 15 min after anti-IgE challenge, there was an abrupt inhibition of histamine release.

Comparison of DDA with other P-site agonists

The comparative effects of 100–1000 μM concentrations of DDA, 2'-deoxyadenosine (2DA) and 9-β-D-arabinofuranosyladenine (ARA-A) against anti-IgE and A23187-induced histamine release from human basophils was assessed in three experiments (Fig. 4).

When added 15 min before anti-IgE challenge, all three adenosine analogues showed concentration-related inhibition of histamine release, the potency order being DDA > 2DA > ARA-A. The IC_{25} values for DDA and 2DA were 60 and 372 μM respectively and that of ARA-A, estimated by extrapolation of the concentration-response line, was in

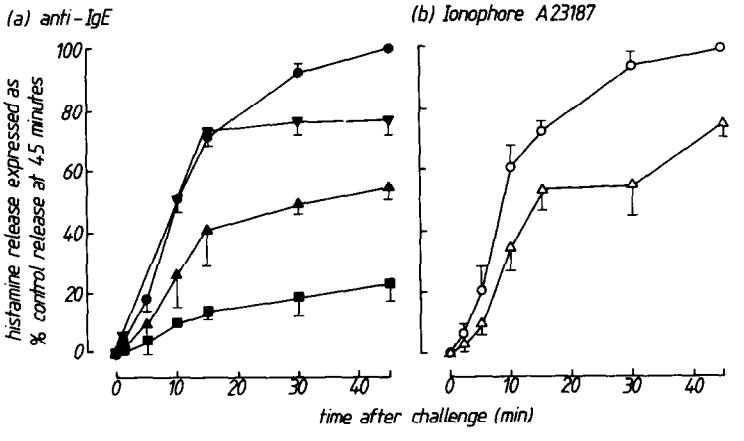

Fig. 3. The effect of DDA (1000 μM) on the time course of histamine release from human basophil leucocytes. (a) DDA was added 15 min before (■), simultaneously with (▲), or 15 min after (▼) anti-IgE challenge (●). (b) DDA was added simultaneously with (△) ionophore A23187 (○). The results are expressed as the mean percentage of the histamine release produced by 45 min after challenge ±S.E.M. of three experiments. Net histamine release at 45 min produced by a 1/1000 dilution of anti-IgE was 21.7 ± 3.2% and by 1 μM ionophore A23187 was 43.2 ± 6.5%.

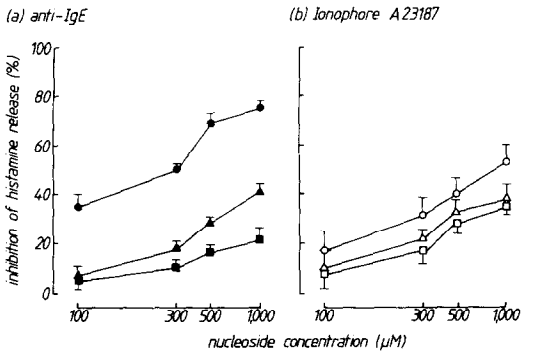

Fig. 4. The effect of DDA (●), 2′-deoxyadenosine (▲) and 9-β-D-arabinofuranosyladenine (■) on histamine release from human basophil leucocytes induced by anti-IgE (closed symbols) and ionophore A23187 (open symbols). Results are expressed as the mean ± S.E.M. inhibition produced in three experiments in which the modified nucleosides were added to the leucocyte preparations 15 min prior to anti-IgE challenge (net histamine release was 47.2 ± 6.1% and spontaneous release was 5.7 ± 0.8%) or simultaneously with A23187 (net histamine release was 38.0 ± 6.1% and spontaneous release was 3.7 ± 1.1%).

the order of 2000 μM. Although not shown in Fig. 4, DDA and 2DA also produced concentration-related inhibition of histamine release when added simultaneously with anti-IgE, the IC_{25} values being 181 and 495 μM respectively. ARA-A produced no significant inhibition under these conditions. With addition of nucleosides simultaneously with A23187, the same order of potency, DDA > 2DA > ARA-A, was observed but the differences were less marked, the IC_{25} values being 174, 291 and 355 μM respectively.

Investigations in the site of action of DDA

Effect of theophylline and dipyridamole on DDA activity. To determine whether the inhibitory effects of DDA result from its interaction with cell surface purinoceptors, leucocytes were preincubated for 5 min with the purinoceptor antagonist theophylline prior to DDA addition in three experiments (Table 1). At 50 μM, a concentration which inhibits purinoceptor stimulation in basophils but does not inhibit basophil histamine release in the absence of adenosine [5], theophylline did not reduce the inhibitory effects of DDA on either anti-IgE or A23187 induced histamine release.

Conversely, pretreatment of leucocytes with the purine uptake inhibitor, dipyridamole (1 μM), for 5 min prior to DDA addition, significantly inhibited the effects of DDA (Table 2). With addition of DDA 15 min before anti-IgE challenge, dipyridamole produced a 16-fold shift to the right of the DDA concentration-response curve, the IC_{25} values of DDA in the absence and presence of uptake inhibitor being 34.9 and 573 μM respectively. With simultaneous addition of DDA and anti-IgE, the concentration-response line was shifted 5-fold to the right by dipyridamole, the IC_{25} values being 190 and 948 μM. Similar calculations were not made for DDA added 15 min after anti-IgE or A23187 challenge as the concentration-response lines were not parallel and inhibition of histamine did not exceed 25%.

Effect of homocysteine on DDA activity. Adenosine and some of its analogues may condense intracellularly with homocysteine for form a product which inhibits *S*-adenylmethionine-dependent methylation of phospholipids [18] and cyclic AMP phosphodiesterase, both of which have been suggested to inhibit basophil histamine release. To test whether DDA acts by this mechanism, leucocyte preparations were incubated with 100 μM homocysteine thiolactone or HBSS for one hour before the addition of 500 μM DDA in three experiments. In no case did homocysteine significantly increase the inhibitory effects of DDA. Inhibition of histamine

Table 1. Effect of theophylline (50 μM) on inhibition of histamine release from human basophils produced by DDA

Time of addition of DDA	DDA (μM)	Inhibition of histamine release (%) DDA alone	DDA + Theo
Anti-IgE			
15 min pre	100	38.8 ± 2.2	34.9 ± 5.7
	500	68.0 ± 4.1	65.9 ± 2.0
	1000	81.2 ± 4.7	78.4 ± 2.9
simultaneous	100	15.1 ± 4.9	20.1 ± 2.9
	500	38.7 ± 3.1	34.3 ± 2.4
	1000	39.1 ± 3.6	41.3 ± 3.3
15 min after	100	8.7 ± 0.9	8.7 ± 1.3
	500	18.4 ± 0.7	18.1 ± 1.5
	1000	38.9 ± 2.9	35.7 ± 6.9
A23187			
simultaneously	100	6.7 ± 1.5	8.7 ± 0.6
	500	14.7 ± 3.1	16.2 ± 1.8
	1000	28.2 ± 1.5	26.6 ± 3.7

Each result is the mean ±S.E.M. of three experiments where the leucocytes were preincubated with theophylline for 5 min prior to the addition of DDA. Analysis of the data by paired *t*-test revealed no significant effect was produced by theophylline.

Table 2. The effect of dipyridamole on DDA mediated inhibition of histamine release from human basophils

Time of addition of DDA	DDA (μM)	Inhibition of histamine release (%) DDA alone	DDA + dipy
Anti-IgE			
15 min pre	100	45.2 ± 2.4	6.5 ± 4.6*
	500	68.2 ± 2.9	20.0 ± 1.2*
	1000	83.5 ± 2.0	38.8 ± 5.4*
simultaneously	100	15.7 ± 8.9	2.4 ± 2.4*
	500	39.4 ± 9.9	20.2 ± 6.9*
	1000	52.9 ± 8.5	23.9 ± 2.1*
15 min post	100	9.1 ± 1.0	−0.9 ± 2.8*
	500	15.1 ± 0.2	0.9 ± 2.9*
	1000	20.8 ± 0.6	10.4 ± 4.9*
A23187			
simultaneously	100	6.8 ± 1.5	3.6 ± 0.4
	500	14.7 ± 3.1	5.1 ± 0.7*
	1000	28.8 ± 1.5	18.9 ± 1.7*

Each result is expressed as the mean ±S.E.M. of three experiments. Dipyridamole (dipy, 1 μM) was added 5 min before DDA. Histamine release induced by anti-IgE was 28.1 ± 4.5% and by A23187 was 74.6 ± 3.7%. * indicates that the inhibitory effect of DDA was significantly ($P < 0.05$) reversed by dipyridamole as calculated by paired *t*-test.

release by DDA in the absence and presence of homocysteine respectively were: 59.7 ± 2.4% and 63.2 ± 4.5% with DDA addition 15 min before anti-IgE challenge; 26.7 ± 6.2% and 22.7 ± 2.2% with simultaneous addition of DDA and anti-IgE; 26.2 ± 5.4% and 24.2 ± 2.6% with addition of DDA 15 min after anti-IgE challenge; and, 27.1 ± 2.4% and 25.7 ± 6.0% with DDA added simultaneously with A23187 challenge.

Is inhibition of adenylate cyclase activity the likely mechanism of action of DDA?

Effect of DDA on anti-IgE-induced cyclic AMP changes. Challenge of human leucocytes containing 40–70% basophils [17] with anti-IgE (1/3000 dilution) in three experiments caused an early monophasic rise in cyclic AMP which reached a maximum of 165 ± 10% above baseline 45 sec after challenge (Fig. 5). This was followed by a smaller rise reaching 55 ± 13% at 2 min after which cyclic AMP levels returned to baseline. Histamine release in these experiments was 13.2 ± 2.4% measured at 45 min. In one experiment, preincubation of leucocytes with 500 μM DDA for 15 min before challenge, completely abolished the early anti-IgE-induced rise in cyclic AMP, a transient fall being observed instead (Fig. 5). A small maintained rise in cyclic AMP levels of 33% above baseline was seen between 5 and 15 min after challenge. Histamine release at 45 min was 6.1%, a reduction of 46% compared with controls.

Effects of manganese and islet activating protein on DDA activity. Substitution of extracellular magnesium by manganese renders adenylate cyclase more responsive to inhibition by P-site agonists [19, 20]. Conversely, islet activating protein (IAP), purified from *Bordetella pertussis*, reduces receptor-mediated inhibition of adenylate cyclase activity by inactivating the N_i protein of the cyclase [21, 22]. If DDA reduces basophil histamine release by inhibiting adenylate cyclase activity then its effects should be enhanced by manganese and reduced by IAP.

The effect of manganese was tested in three experiments in which manganese, 1 mM, was substituted for magnesium in all washing and challenge stages. Although not effecting histamine release induced by anti-IgE (1/1000 dilution) or A23187 (1 μM) in the

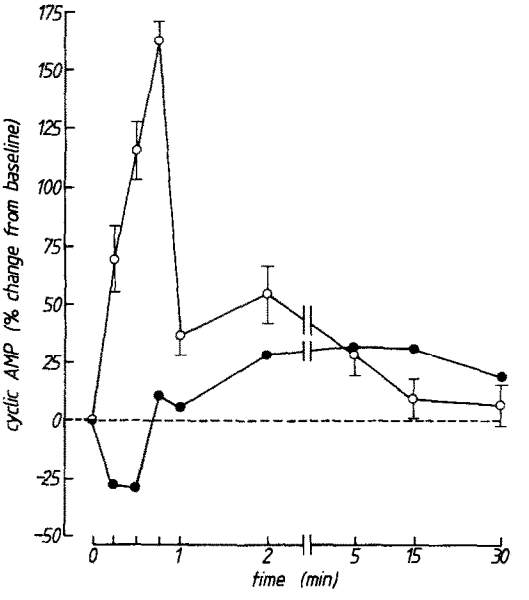

Fig. 5. The effect of DDA on anti-IgE induced changes in cyclic AMP levels of basophil rich human leucocyte preparations. Cells were challenged with 1/3000 dilution of anti-IgE. Results are mean ± S.E.M. for three experiments using untreated cells(○) in which net histamine release at 45 min was 13.2 ± 2.4%. In one experiment where cells were preincubated with 500 μM DDA for 15 min before challenge (●) net histamine release at 45 min was 6.1%. Baseline cyclic AMP levels were 0.78 ± 0.02 pmol/10^6 nucleated cells.

absence of DDA, manganese caused a significant ($P < 0.05$) enhancement of the inhibitory effects of DDA added simultaneously with both secretagogues (Fig. 6). With anti-IgE challenge, manganese caused a four-fold potentiation of DDA activity, the IC_{25} in the presence of manganese being 121 μM whilst that in HBSS containing magnesium was 473 μM. With A23187 challenge, manganese caused a two-fold shift in the concentration–response line, the IC_{25} values being 246 μM and 500 μM in the presence of manganese and magnesium respectively.

In an initial experiment to determine an appropriate concentration of IAP to use in interactive studies with DDA, leucocytes were preincubated for two hours with 50–2000 μg/ml IAP before challenge with anti-IgE or A23187. With anti-IgE challenge IAP caused a concentration-related inhibition of histamine release with an IC_{25} of 118 μg/ml whereas with A23187, IAP concentration in excess of 500 μg/ml were required to produce inhibition. In subsequent experiments leucocytes were preincubated at 37° for 2 hr with 100 ng/ml IAP. In three experiments, IAP alone inhibited anti-IgE induced histamine release by 18.4 ± 3.6% ($P < 0.05$) but significantly reversed the effects of DDA (Table 3). In the presence of IAP the IC_{25} for DDA was 368 μM whereas in its absence it was 124 μM, a threefold shift in the concentration–response line. Incubation of leucocytes with IAP alone did not significantly effect the secretory response to A23187 but significantly ($P < 0.05$) reduced the inhibitory effects of DDA (Table 3). In the absence of IAP, the IC_{25} for DDA was 752 μM but in its presence DDA produced only 14.1% inhibition at 1000 μM, the maximum concentration used.

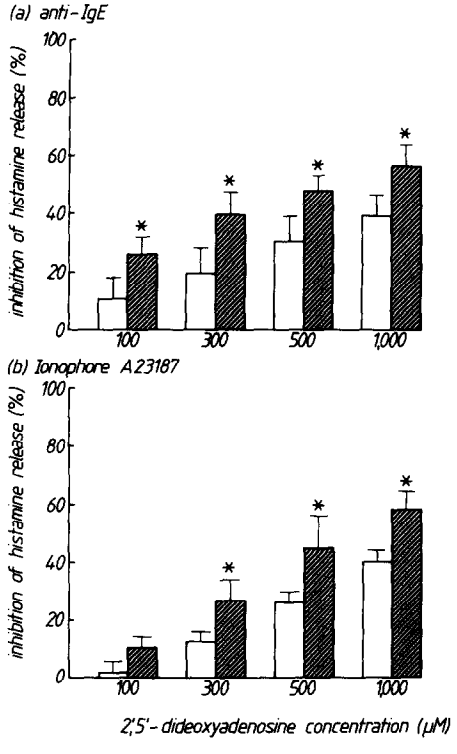

Fig. 6. The effect of manganese on DDA mediated inhibition of histamine release from human basophil leucocytes. The leucocytes were preincubated and challenged in HEPES buffered physiological saline containing either 0.5 mM magnesium (open columns) or with HEPES buffered physiological saline containing 1 mM manganese. DDA was added at the same time as the secretagogues. Each result is the mean ± S.E.M. of three experiments in which the cells had been challenged with a 1/1000 dilution of anti-IgE (net histamine release was 38.7 ± 3.8% and spontaneous histamine release was 8.9 ± 2.2%) or A23187 (1 μM, net histamine release was 70.9 ± 8.3% and spontaneous release was 7.5 ± 0.7%). * Indicates significantly ($P < 0.05$) different from the corresponding control.

Table 3. The effect of islet activating protein from *Bordetella pertussis* (100 ng/ml) on inhibition by DDA of anti-IgE and ionophore A23187 induced histamine release from human basophils

DDA (μM)	Net histamine release (%) DDA	DDA + IAP
Anti-IgE Challenge		
0	33.4 ± 4.4	28.4 ± 4.5*
100	25.5 ± 1.2	21.8 ± 1.3*
300	13.7 ± 3.9	20.3 ± 2.8*
500	9.4 ± 2.7	16.9 ± 2.8*
1000	8.8 ± 1.8	13.0 ± 1.6*
A23187 Challenge		
0	39.4 ± 8.5	42.0 ± 8.7
100	38.1 ± 6.3	41.8 ± 8.8
300	33.5 ± 6.5	38.6 ± 6.3*
500	30.0 ± 6.3	38.0 ± 8.2*
1000	26.4 ± 6.8	36.1 ± 8.3*

Each result is the mean ±S.E.M. of three experiments where the leucocytes were preincubated for 2 hr in the presence or absence of IAP (100 ng/ml) and DDA added simultaneously with anti-IgE (1/1000 dilution) or A23187 (1 μM). IAP itself did not increase spontaneous release.

* Indicates significantly ($P < 0.05$) different from the corresponding value in the absence of IAP assessed by paired *t*-test.

Effect of nicotinic acid on histamine release. If DDA inhibits histamine release by inhibiting adenylate cyclase activity, then its action should be mimicked by nicotinic acid which also causes a receptor mediated inhibition of the cyclase [23–25]. This hypothesis was tested in two experiments in which nicotinic acid, 0.1 to 10,000 μM, was added to leucocytes simultaneously with anti-IgE or A23187. With both secretory stimuli, nicotinic acid caused a concentration-related inhibition of histamine release reaching a maximum of 41.3% ($P < 0.01$) and 38.2% ($P < 0.01$) respectively. The IC_{25} values for nicotinic acid were 204 μM and 446 μM respectively for inhibiting secretion induced by anti-IgE and A23187.

DISCUSSION

2',5'-Dideoxyadenosine (DDA) has been shown to inhibit IgE-dependent and calcium ionophore A23187-induced histamine release from human basophil leucocytes with different time-related characteristics. The inhibition produced by DDA was inversely related to histamine release. When added simultaneously with challenge, DDA was approximately twice as potent in inhibiting anti-IgE induced histamine release when compared with release stimulated by A23187. Other P-site active analogues of

adenosine, 2DA and ARA-A, were less effective than DDA. Studies with theophylline, dipyridamole and homocysteine thiolactone suggest DDA to have an intracellular action which is not associated with inhibition of *S*-adenosylmethionine-dependent methylation. The findings that IgE-dependent changes in cyclic AMP levels are abolished by DDA, that manganese potentiates and IAP reduces its effects and that nicotinic acid mimics its effects all suggest that inhibition of adenylate cyclase or modification of Ni protein activity to be the likely mechanism of action of DDA.

Experiments in which DDA was added to leucocyte preparations at times from 15 min before anti-IgE challenge to 15 min after challenge showed that the inhibitory efficacy of the drug was enhanced with increasing preincubation time. With addition after challenge, DDA appeared to lose its efficacy, but this was not strictly correct as the basophils had already secreted a proportion of their histamine before DDA was added. For example, time course experiments showed that, by 15 min, 71% of the releasable histamine had been secreted. Thus, the 18.1% inhibition of release observed with addition of DDA 15 min after anti-IgE challenge could be interpreted as a 62% inhibition of that 29% of histamine yet to be released. With A23187 challenge, DDA was ineffective when preincubated with cells but simultaneous addition of drug and secretagogue resulted in a significant inhibition of release. Again, efficacy appeared to wane when DDA was added after A23187. However, when appropriate corrections were made for histamine already released, inhibition remained constant at 18–20%.

Concentration–response studies showed a linear relationship between DDA concentration and inhibition of histamine release in all experiments. In membrane preparations, the IC_{50} for inhibition of adenylate cyclase activity by DDA is in the range 1–10 μM. However, the IC_{25} values for inhibiting histamine release from human basophils were in excess of 100 μM, concentrations consistent with those observed in human and rodent mast cells [2, 10, 11]. This difference probably reflects the poor cellular uptake of 2′-deoxy-nucleosides which are not substrates for the low affinity carrier system through cell membranes [26].

Comparison of DDA with 2′-deoxyadenosine (2DA) and 9-β-D-arabinofuranosyladenine (ARA-A) showed the potency order DDA > 2DA > ARA-A in inhibiting basophil histamine release induced by anti-IgE and A23187. This order of potency agrees with that found for inhibition of bovine thyroid membrane and cytosolic adenylate cyclase [27] but differs from results using human platelet, mouse I-10 Leydig tumor cells and rat cerebral membranes where the order was DDA > ARA-A > 2DA [9, 28]. Furthermore, the inhibitory effects of ARA-A in basophils contrasts with results using rat mast cells where ARA-A is ineffective against histamine release induced by both anti-IgE and A23187 [10].

The site of action of DDA was explored by the use of theophylline, dipyridamole and homocysteine thiolactone. An interaction of DDA with cell surface A_1 or A_2-purinoceptors is unlikely as theophylline, a competitive antagonist of these receptors did not affect its activity. However, dipyridamole, a competitive inhibitor of the high affinity system for purine uptake [29], significantly reduced the activity of DDA. This is highly suggestive of an intracellular mechanism of DDA. An inhibitory effect of DDA mediated by inhibition of *S*-adenyosylmethionine-dependent methylation reactions is unlikely as these require the intracellular formation of the nucleoside-homocysteine derivative [18]. The absence of a hydroxyl group at the 5′ position of the ribose ring precludes formation of such a derivative. Furthermore, homocysteine thiolactone, which enhances the formation of nucleotide-homocysteine derivatives [18], failed to increase the inhibitory effect of DDA against either anti-IgE or A23187 induced histamine release.

Inhibition of adenylate cyclase activity by DDA in many *in vitro* systems has been reported to result from an interaction of the nucleotide with P-sites on the cytoplasmic surface of the cell membrane [28]. In basophil-rich human leucocyte preparations, the early rise in intracellular cyclic AMP levels induced by anti-IgE challenge was blocked by DDA in parallel with a 45% reduction in histamine release. Such a relationship has previously been reported for rat mast cells [7]. Therefore, a possible explanation for the effects of DDA on basophil histamine release is an inhibition of adenylate cyclase activity. Although the exact location of the P-site is not known, it is thought to be intimately associated with the catalytic protein of adenylate cyclase as the presence of a functional guanine nucleotide binding regulatory protein (N protein) is required for the full expression of P-site agonists [30, 31].

The ability of adenosine to modify adenylate cyclase activity, like that of other hormones, is modulated by divalent cations. Manganese in particular enhances the inhibition of adenylate cyclase produced by A_1-purinoceptor stimulation and P-site agonists [20] by promoting the formation of a highly stable complex between the receptor protein, the inhibitory Ni guanine nucleotide binding regulatory protein and the adenylate cyclase catalytic subunit. Manganese appears to be 10 times more potent than magnesium in producing this effect [19, 20]. Marone *et al.* [32] have demonstrated in human lymphocytes that incubation with 1 μM manganese increases the ability of DDA to inhibit agonist induced activation of adenylate cyclase. The observation that replacement of magnesium in the incubation medium by 1 mM manganese significantly increased the magnitude of the DDA mediated inhibition of anti-IgE and A23187 induced histamine release is consistent with enhancement of P-site activity.

IAP stabilizes the inhibitory (N_i) subunit of adenylate cyclase guanine nucleotide-binding regulatory protein [21]. Preincubation of leucocytes for 2 hr with 100 ng/ml IAP significantly reversed the inhibitory actions of DDA agonist anti-IgE and A23187 induced histamine release suggesting that DDA inhibition is mediated by a suppression of adenylate cyclase activity. If this is so, then other agents which reduce adenylate cyclase activity should also inhibit histamine release. Nicotinic acid, at high concentrations, inhibits adenylate cyclase activity by stimulating GTPase activity in the regulatory subunit

[23–25]. This agent, whose activity is also reversed by IAP [25], produced a concentration-related inhibition of leucocyte histamine release and, like DDA, was more effective against anti-IgE stimulation than A23187-induced release.

A23187 stimulation of mediator release is not associated with an early elevation of cyclic AMP, a finding which has led to the suggestion that cyclic AMP is not involved in ionophore-induced secretion [33]. However, the experiments with manganese IAP and nicotinic acid suggest that the DDA mediated inhibition of A23187-induced histamine release could be produced by suppression of adenylate cyclase activity. The observation that time courses for DDA mediated inhibition of anti-IgE and A23187-induced histamine release from basophils are markedly different is not, however, easily compatible with a unified concept of adenylate cyclase inhibition.

As manganese and IAP both modify hormone-adenylate cyclase interaction at the level of the Ni-proteins the observations that these agents can modify DDA-mediated inhibition of histamine release suggests that DDA could act via an interaction with a guanine nucleotide binding protein (GNBP) not associated with adenylate cyclase. As has been suggested in other cell types [34], the basophil may contain a whole family of GNBPs each having a different function in the release process. There is some evidence to suggest that GTP and GNBPs may be involved in drug hormone induced activation of polyphosphoinositide phosphodiesterase and subsequent accumulation of inositol trisphosphate and diacylglycerol [35], protein kinase C activation [36], calcium translocation [37] and phospholipid transmethylation reactions [38, 39]. All these biochemical processes have been implicated in the initiation and maintenance of mediator release reactions and are therefore potential sites where DDA might inhibit release.

In summary, the P-site agonist DDA inhibits both immunologically and non-immunologically stimulated histamine secretion from human basophil leucocytes. The site at which the inhibition is produced is intracelluar. From experiments with agents that modify the activity of the inhibitory guanine nucleotide binding subunits (N_i) of adenylate cyclase it can be concluded that this is a possible site of DDA's activity. However, the possibility that DDA may be acting at other guanine nucleotide binding subunits not functionally related to adenylate cyclase cannot be ruled out.

REFERENCES

1. T. Ishizaka and K. Ishizaka, *Prog. Allergy* **34**, 188 (1984).
2. P. A. Hillyard, A. T. Nials, I. F. Skidmore and C. J. Vardey, *Br. J. Pharmac.* **83**, 337 (1984).
3. P. J. Hughes, S. T. Holgate and M. K. Church, *Biochem. Pharmac.* **33**, 3847 (1984).
4. G. Marone, S. R. Findlay and L. M. Lichtenstein, *J. Immunol.* **123**, 1473 (1979).
5. M. K. Church, S. T. Holgate and P. J. Hughes, *Br, J. Pharmac.* **80**, 719 (1983).
6. D. L. Marquardt, C. W. Parker and T. J. Sullivan, *J. Immunol.* **120**, 871 (1978).
7. S. T. Holgate, R. A. Lewis and K. F. Austen, *Proc. natn. Acad. Sci. U.S.A.* **77**, 6800 (1980).
8. M. K. Church and P. J. Hughes, *Br. J. Pharmac.* **85**, 3 (1985).
9. C. Londos and J. Wolff, *Proc. natn. Acad. Sci. U.S.A.* **74**, 5482 (1977).
10. D. S. Burt and D. R. Stanworth, *Biochem. Pharmac.* **32**, 2729 (1983).
11. M. Nishibori, K. Shimamura, H. Yokoyama, K. Tsutsumi and K. Saeki, *Archs. Int. Pharmacodyn.* **265**, 17 (1983).
12. A. Leoutsakos, A. Truneh and F. L. Pearce, *Agents Actions* **16**, 126 (1985).
13. A. F. Welton and B. A. Simko, *Biochem. Pharmac.* **29**, 1085 (1980).
14. M. K. Church, G. J-K. Pao and S. T. Holgate, *J. Immunol.* **129**, 2116 (1982).
15. L. M. Lichtenstein and A. G. Osler, *J. exp. Med.* **120**, 507 (1964).
16. D. P. Evans, J. A. Lewis and D. S. Thomson, *Life Sci.* **12**, 327 (1973).
17. P. J. Hughes, S. T. Holgate, S. Roath and M. K. Church, *Biochem. Pharmac.* **32**, 2557 (1983).
18. P. M. Ueland, *Pharm. Rev.* **34**, 223 (1982).
19. J. Premont, G. Fuillon and J. Bockaert, *Biochem. biophys. Res. Commun.* **90**, 513 (1979).
20. S-M. H. Yeung, L. T. Frame, J. C. Ventor and D. M. F. Cooper, in *Purines: Pharmacology and Physiological Roles* (Ed. T. W. Stone), p. 203. Macmillan, London (1985).
21. M. Ui, *Trends Pharm. Sci.* **14**, 223 (1983).
22. A. C. Wardlaw and R. Parton, *Pharm. Ther.* **19**, 1 (1983).
23. K. Aktories, K. H. Jacobs and G. Schultz, *FEBS Letters* **115**, 11 (1980).
24. K. Aktories and K. H. Jacobs, *Naunyn-Schmiedebergs Archs. Pharmac.* **318**, 241 (1982).
25. K. Aktories, G. Schultz and K. H. Jacobs, *FEBS Letters* **156**, 88 (1983).
26. J. W. Daly, *J. Med. Chem.* **25**, 197 (1982).
27. J. Wolff, C. Londos and G. H. Cook, *Archs. Biochem. Biophys.* **191**, 161 (1978).
28. Y. Nimit, J. Law and J. W. Daly, *Biochem. Pharmac.* **31**, 3279 (1982).
29. K. Turnheim, B. Plank and N. Kolossa, *Biochem. Pharmac.* **27**, 2191 (1978).
30. J. Wolff, C. Londos and D. M. F. Cooper, *Adv. Cyc. Nuc. Res.* **14**, 199 (1981).
31. H. Shimizu, in *Physiology and Pharmacology of Adenosine Derivatives* (Eds. J. W. Daly, Y. Kuroda, J. W. Phillis, H. Shimizu and M. Ui), p. 31. Raven Press, New York (1983).
32. G. Marone, R. Petracca and S. Vigorita, *Int. Archs. Allergy Appl. Immunol.* **77**, 259 (1985).
33. L. M. Lichtenstein, *J. Immunol.* **114**, 1692 (1975).
34. M. Rodbell, in *Drug Action: Dynamic Processes in Membranes and Cells Versus the Drug Receptor Concept. Alfred Benzon Symposium 22* (Eds. J. S. Schou, A. Geisler and S. Norn). Munksgaard, Copenhagen (in press).
35. S. Cockcroft and B. D. Gomperts, *Nature, Lond.* **314**, 534 (1985).
36. M. Volpi, P. H. Naccache, T. P. Molski, J. Sefcyk, K-H. Huang, M. L. Marsh, J. Munoz, E. L. Becker and R. I. Sha'afi, *Proc. natn. Acad. Sci. U.S.A.* **82**, 2708 (1985).
37. T. P. Molski, P. H. Naccache, M. L. Marsh, J. Kermode, E. L. Becker and R. I. Sha'afi, *Biochem. biophys. Res. Commun.* **121**, 644 (1984).
38. K. Hashizume, M. Kobayashi and K. Ichikawa, *Biochem. biophys. Res. Commun.* **114**, 425 (1983).
39. J. S. Zawad and F. C. Brown, *Biochem. Pharmac.* **33**, 3799 (1984).