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REGULATORY ROLE OF ADENOSINE IN ANTIGEN-INDUCED HISTAMINE RELEASE FROM THE LUNG TISSUE OF ACTIVELY SENSITIZED GUINEA PIGS

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Abstract—Adenosine potentiates the *in vitro* antigen-induced release of histamine from chopped lung tissue prepared from a guinea pig actively sensitized to egg albumin. By studying the activity of a variety of adenosine analogs, the potentiation process in lung has been characterized as occurring through an 'R' adenosine receptor site. Similar studies on ionophore A23187-induced release of histamine from purified rat peritoneal mast cells have demonstrated that an 'R' adenosine receptor site is also associated with adenosine potentiation in this system. Since adenosine inhibits adenylate cyclase activity in a homogenate of guinea pig lung, and since decreases in cyclic AMP levels are thought to be associated with mediator release, the regulatory site involved in the enzyme inhibition process was also characterized to determine if, in lung, the ability of adenosine to inhibit adenylate cyclase is associated with potentiation of mediator release. It was found, however, that inhibition of adenylate cyclase occurs through a 'P' site, suggesting that the two processes are not related phenomena. Theophylline blocks the effect of adenosine on the antigen-induced release of histamine from guinea pig lung. This is in accord with previous data demonstrating that theophylline is an 'R' site antagonist. This observation also supports the idea that part of the pharmacological action of theophylline in human bronchial asthma may be through inhibition of the ability of adenosine to potentiate mediator release.

Adenosine has been demonstrated to have an important regulatory role in the metabolic processes carried out by a wide variety of cell types including adipocytes [1–3], adrenal cells [4–6], brain cells [7–10], lymphocytes [11, 12], chondrocytes [13], bone cells [14] and platelets [15, 16]. Marquardt *et al.* [17] have demonstrated recently that adenosine potentiates the release of histamine from purified rat peritoneal mast cells. They observed that not only is adenosine a potent modulator of release but that this effect can be blocked by low concentrations of theophylline (1–10 μM), concentrations in the range previously found to suppress allergic asthmatic reactions [18]. They suggest, on the basis of this information, that this property of theophylline may account, in part, for its therapeutic effectiveness in treating asthmatics. Since these studies were performed using purified mast cells under 'artificial' release conditions (release was induced using either ionophore, compound 48/80, concanavalin A or anti-IgE), it is not clear if adenosine has a regulatory role in mediator release under more physiological conditions, such as in sensitized lung tissue, where antigen is used to induce release. The experiments reported in this paper examine this question by demonstrating that adenosine potentiates antigen-induced histamine release from sensitized guinea pig lung.

The mechanism by which adenosine potentiates mediator release from mast cells or lung tissue is not known. In many cells, this nucleoside is believed to act by modulating cyclic AMP levels through interaction with regulatory sites associated with adenylate cyclase systems. In some cells, modulation appears to occur by activation of this enzyme system and, in others, by inhibition [1, 19–26]. Such observations have lead Londos and Wolff [27] to propose that adenosine modulates adenylate cyclase, and perhaps cellular metabolic processes, through two types of regulatory sites—an 'R' site, so named because it does not recognize adenosine derivatives which have modifications in their ribose component, and a 'P' site, which does not recognize derivatives with alterations in the purine components of the nucleoside. Depending upon the type of cell, 'R' sites are either activating sites or inhibitory sites, while 'P' sites seem to always be inhibitory [27, 28]. In mast cells, Marquardt *et al.* [17] have reported that adenosine accentuates the decrease in cyclic AMP levels normally associated with histamine release, perhaps by inhibition of adenylate cyclase. These observations, and those made by Weinryb and Michel [25] that adenosine inhibits the adenylate cyclase system assayable in guinea pig lung, led us to investigate whether the ability of adenosine to inhibit lung adenylate cyclase might be related to its ability to potentiate mediator release. Therefore, we characterized the adenosine sites involved in these processes as being either 'R' or 'P' sites by use of a variety of adenosine analogs [27]. The results of this study demonstrated that in guinea pig lung the regulatory site involved in the potentiation of mediator release is an 'R' site. This may be a general property of the adenosine sites associated with allergic mediator release since a similar characterization of the adenosine site associated with the release potentiation process in rat peritoneal cells demonstrated that it, too, is an 'R' site. In contrast, the adenosine site involved in inhibition of guinea pig lung adenylate cyclase is a 'P' site. Thus, in lung tissue we were not

able to associate the effect of adenosine on the mediator release phenomenon with an effect of the nucleoside on the adenylate cyclase activity assayable in a lung homogenate.

MATERIALS AND METHODS

Materials

The following chemicals were purchased from the Sigma Chemical Co., St. Louis, MO: chicken egg albumin (Grade V), adenosine, adenosine 5′-triphosphate (A6144), creatine phosphate, creatine phosphokinase, dithiothreitol, adenosine 3′,5′-cyclic monophosphate, histamine dihydrochloride, 2′-deoxyadenosine, 2-chloroadenosine and adenine. N^6-Methyladenosine, 2-methyladenosine and 9-β-D-arabinofuranosyladenine were obtained from ICN, Irvine, CA. PL Biochemicals, Milwaukee, WI, supplied the 5′-deoxyadenosine, 2′,5′-dideoxyadenosine and formycin B. N^6-Phenylisopropyladenosine was obtained from Boeringer-Mannheim, Indianapolis, IN, and *o*-phthalaldehyde was obtained from Eastman, Rochester, NY. Ionophore A23187 was a gift of the Lilly Research Laboratories, Indianapolis, IN.

Methods

Histamine release from chopped guinea pig lung. The method used to study antigen-induced histamine release from chopped guinea pig lung was an adaptation of that described by Hitchcock [29]. Male guinea pigs (200–250 g) from the Hartley strain were obtained from Charles River (Wilmington, MA). These animals were actively sensitized with a single injection, i.p., of 10 mg egg albumin in 1 ml of 0.9% NaCl. Twenty-eight to forty-five days following sensitization, the animals were stunned, decapitated and exsanguinated, and the lungs were removed. The lungs were immediately placed in chilled Tyrodes solution, dissected free from the major airways and blood vessels, and chopped into 1 mm^3 fragments using a McIlwaine tissue chopper. After chopping, the lung fragments from several guinea pigs were combined, mixed, filtered through cheesecloth, and washed free of blood with additional Tyrodes solution. The lung fragments were weighed into 150 mg portions. Each histamine release assay was conducted in a total volume of 5 ml of Tyrodes solution at 37°, utilizing air as the gas phase (essentially the same release was obtained utilizing either air or a mixture of 95% O_2 and 5% CO_2 as the gas phase). All assays were run in quadruplicate and the lung tissue was normally prewarmed at 37° for the periods described in the figure and table legends prior to the addition of antigen (egg albumin) to start the release reaction. Release was carried out for a period of 10 min, utilizing 40 μg/ml egg albumin. Mediator release was complete during this time period. Reactions were terminated by filtration through Whatman No. 1 filter paper. The filtrate containing the released histamine was acidified to 0.4 N $HClO_4$, and precipitation of protein contaminants was carried out at 0–4° for at least one hr. The samples were then centrifuged at 500 *g* for 10 min, and the histamine content of the supernatant fraction was quantitated. utilizing an automated fluorometric assay system employing a Technicon Autoanalyzer II [30]. The various adenosine analogs used in the studies reported here did not interfere with the fluorometric histamine analysis. In all experiments, the total histamine content of the lung was determined by homogenizing 150 mg of chopped lung in 5 ml of Tyrodes. These samples were then boiled for 10 min and centrifuged at 500 *g* for 10 min to remove the tissue fragments. The supernatant fraction was acidified to 0.4 N $HClO_4$, as described above, and subsequently analyzed for histamine content. Spontaneous release from the tissue was ascertained by carrying 150 mg samples of lung tissue, in the absence of antigen, through the complete assay procedure. The per cent spontaneous release averaged 3.2 ± 0.4 ($N = 15$). The per cent release induced by antigen was calculated by subtracting the per cent spontaneous release from the per cent release obtained in the presence of antigen. As described by Hitchcock [29], we observed that there was a concentration-dependent increase in the per cent histamine release with increasing antigen concentration and that the response range was very broad (perhaps due to penetration problems in getting the antigen into the lung fragments). At 40 μg/ml of egg albumin, the average per cent release of histamine in fifteen experiments was 24.1 ± 0.9. To determine the per cent potentiation of histamine release induced by adenosine and adenosine analogs, the per cent release obtained in the presence of antigen alone and antigen with adenosine or analog was determined. The difference in these values divided by the per cent release obtained with antigen alone was the per cent potentiation.

Ionophore A23187-induced histamine release from purified rat peritoneal mast cells. Rat peritoneal mast cells were purified by a modification of the method of Sullivan *et al.* [31]. Hank's balanced salt solution (buffered to pH 6.8 with 0.005 M sodium phosphate buffer) was used throughout the procedure (this buffer will subsequently be termed 'stock buffer'). All manipulations were carried out at room temperature. Ten Sprague–Dawley rats (190–210 g) were fasted overnight prior to being killed. The peritoneal cells were removed from the abdomen of the rats by lavage with 10 ml of stock buffer and isolated by centrifugation at 50 *g* for 7 min. The resulting pellets were resuspended in a total volume of 10 ml of stock buffer (1 ml/rat used in the isolation) and 2 ml of this suspension was layered over 4 ml of a 38% bovine serum albumin (BSA) solution. This BSA gradient was then centrifuged at 150 *g* for 20 min. The mast cells entered the BSA layer during the centrifugation. Contaminating cells in the upper layer of the gradient and banding at the BSA layer were removed with a Pasteur pipet and discarded. The BSA layer was diluted with 5 vol. of stock buffer and centrifuged for 10 min at 150 *g*. The pelleted cells were washed once more by resuspension in stock buffer (10 ml/rat used in the isolation) and recentrifuged at 150 *g*. The cells thus isolated were typically 60–80 per cent mast cells as assessed by toluidine blue staining [32]. For release studies, the

cells were resuspended to a concentration of approximately 100,000 cells/ml.

Ionophore A23187-induced release studies in the presence of adenosine analogs were performed at an ionophore concentration of 3×10^{-8} M. Preliminary experiments indicated that the ionophore, in the concentration range of 10^{-8} to 10^{-7}M, induced a dose-dependent release of histamine. Ionophore (1 mM) was first dissolved in dimethylsulfoxide (DMSO) and subsequently diluted in stock buffer to the final assay concentration of 0.003%. The purified cells were prewarmed in plastic tubes at 37° for 9 min prior to the addition of nucleoside. After 1 additional min, release was initiated by addition of ionophore (the final reaction volume was 2 ml). The release reaction was allowed to proceed for 5 min at 37° and terminated by placing the reaction tubes on ice. The chilled tubes were centrifuged at 500 *g* for 10 min. The supernatant fractions were then adjusted to 0.4 N $HClO_4$, chilled at 0–4° overnight, recentrifuged at 500 *g* for 10 min to remove precipitated protein, and analyzed for histamine content. The total histamine content of the cells and the amount of spontaneous release in the presence of 0.003% DMSO was determined as described for the chopped lung system (see above).

Adenylate cyclase assay. For assay of adenylate cyclase activity and of the effect of adenosine and adenosine analogs on adenylate cyclase activity, a membrane fraction was prepared from the lungs of actively sensitized guinea pigs. All procedures were carried out at 0–4°. The lungs were dissected away from the major airways and blood vessels, cut into small pieces using scissors, and homogenized in 9 vol. of 50 mM Tris–HCl (pH 7.5) containing 1 mM dithiothreitol and 0.25 M sucrose. After filtering the homogenate through cheesecloth, it was centrifuged at 40,000 *g* for 20 min. The pellet obtained from this centrifugation was resuspended in the original volume of homogenizing buffer and stored under N_2 gas at −80°. The enzyme was stable in this form to storage for at least 1 month. Protein determinations on this membrane preparation were performed using the BioRad method [33]. Bovine serum albumin was the protein standard used.

Adenylate cyclase activity was assayed according to the method of Salomon *et al.* [34]. The assay reaction contained, in a volume of 100 μl, 30 mM Tris–HCl (pH 7.5), 5 mM $MgCl_2$, 7 mM creatine phosphate, 2.8 units of creatine phosphokinase, 0.1 mM ethyleneglycolbis-(aminoethylether)tetraacetate (EGTA), 1 mM dithiothreitol, 0.1 mM [α-^{32}P]ATP (20–200 c.p.m./pmole), 0.04 mM cAMP, 50 μg of membrane protein, and varying concentrations of the adenosine analogs described in Table 3 (ranging from 10^{-6} M to 10^{-3} M). The reactions were initiated by the addition of membrane protein and carried out at 37° for 10 min (under these conditions the assay was linear with time and membrane protein). Reactions were terminated by the addition of 100 μl of 'stopping solution' containing 2% sodium dodecylsulfate, 40 mM ATP and 1.4 mM cyclic AMP at pH 7.5. [^{3}H]Cyclic AMP was added to the reaction mixture to monitor recovery during sequential column chromatography through Dowex AG50-WX4 and Alumina WN-3.

RESULTS

Effects of adenosine and adenosine analogs on antigen-induced histamine release from chopped guinea pig lung. When lung fragments from actively sensitized guinea pigs were incubated with adenosine prior to being challenged with antigen (egg albumin), potentiation of the antigen-induced release of histamine was observed (Table 1). In the experiment reported in this table, the lung fragments were preincubated at 37° for 3 min with adenosine prior to challenge of the tissue with antigen. Subsequent experiments indicated similar results could be attained by addition of adenosine simultaneously with antigen. The amount of potentiation observed with adenosine varied from a low of 10 per cent to a high of 35 per cent in twelve experiments (average was 25.1 ± 1.6 per cent). There was no effect of adenosine on histamine release in the absence of antigen.

Analogs with potent 'R' site activity (N^6-methyladenosine, 2-methyladenosine and 2-chloroadenosine [27]) and 'P' site analogs (9-β-D-arabinofuranosyladenine, 5-deoxyadenosine and 2′-deoxyadenosine [27]) were also examined for their ability to potentiate histamine release from lung tissue after antigen challenge. The data presented in Table 1 indicate that the potentiation process appears to occur through 'R' type adenosine sites; that is, only the analogs which were 'R' site agonists mimic the effect of adenosine. This observation has been repeated in four separate experiments. The absolute values for the per cent potentiation observed with the 'R' site analogs varied from experiment to experiment as described above for adenosine, but the qualitative results were always the same in that only 'R' site agonists were active in the potentiation process.

As demonstrated in Fig. 1, the potentiation process induced through the 'R' site was dose related. N^6-Methyladenosine was chosen to examine this phenomenon rather than adenosine itself since this adenosine analog is not metabolized by enzymes such as adenosine deaminase which might be present in the lung fragments [35]. In this regard, it should be noted that inosine, the metabolic product of the deamination of adenosine by adenosine deaminase [35], was not a potentiator of antigen-induced histamine release from the guinea pig lung. Other nucleosides and purine bases which were tested and found to be inactive in this system include guanosine, cytidine, thymidine, uridine, xanthine and hypoxanthine.

Theophylline inhibited the potentiation process induced by the 'R' site adenosine analogs in guinea pig lung. The data in Table 2 demonstrate that there was a dose-dependent decrease in potentiation of release when the reactions were carried out in the presence of N^6-methyladenosine (10^{-5} M) and varying concentrations of theophylline. Theophylline itself, at concentrations up to 10^{-4} M, did not inhibit mediator release in this experiment; therefore, the effect being examined is not due to a general inhibition by theophylline but rather to an effect on the potentiation process.

Effects of adenosine and adenosine analogs on

Table 1. Effects of adenosine and adenosine analogs on the potentiation of histamine release from chopped guinea pig lung*

Analog (100 μM)	Potentiation of histamine release (%)
Adenosine	19.5 ± 2.6 (P<0.001)†
N^6-Methyladenosine	37.7 ± 3.4 (P<0.001)
2-Methyladenosine	29.2 ± 2.0 (P<0.001)
2-Chloroadenosine	33.3 ± 3.4 (P<0.001)
9-β-D-Arabinofuranosyladenine	−2.7 ± 0.3 (NS)
5′-Deoxyadenosine	0.5 ± 2.0 (NS)
2′-Deoxyadenosine	1.3 ± 1.4 (NS)

* Lung tissue from five sensitized guinea pigs was combined for use in this experiment. The chopped lung tissue was prewarmed in Tyrodes solution at 37° for 10 min prior to the addition of adenosine or the indicated adenosine analog. After 3 min the tissue was challenged with 40 μg/ml of egg albumin. Reactions were terminated and histamine release was determined. The per cent histamine release in this experiment was 21.9 ± 0.1 (corrected for a spontaneous release of 4.2 per cent). Per cent potentiation of histamine release was calculated as described in Materials and Methods.

† The values for the per cent potentiation of histamine release are the means ± the S.E. of four determinations. The 'P' values (determined by Student's paired *t*-test) indicate the level of significance of the difference in the release obtained in the presence of adenosine or analog in relation to the release attained with antigen alone. NS = not significant.

ionophore-induced histamine release from rat peritoneal mast cells. Since adenosine has been reported previously to potentiate ionophore-induced histamine release from rat peritoneal mast cells [17], it was of interest to characterize more thoroughly the adenosine site associated with this mediator release system to determine if it, too, was an 'R' site. As can be seen in Table 3, in this system, too, only the analogs which were 'R' site agonists, N^6-methyladenosine, 2-methyladenosine, 2-chloroadenosine and N^6-phenylisopropyladenosine, were significant potentiators of the release process. The regulatory site associated with this system differs from that associated with the guinea pig lung system in one respect, however, in that inosine, at 100 μM, is a significant potentiator of histamine release in purified mast cells (e.g. in the experiment described in Table 3, inosine gave a 132.0 ± 19.5 per cent potentiation). This effect of inosine on purified mast cells which has been described previously by Marquardt

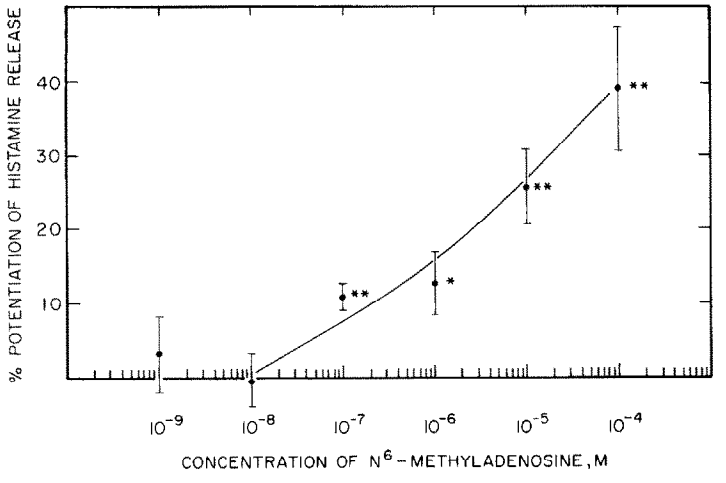

Fig. 1. Dose-related potentiation of antigen-induced histamine release from chopped guinea pig lung by N^6-methyladenosine. Chopped lung tissue from four guinea pigs was combined for use in the experiment. The lung samples were preincubated for 10 min in Tyrodes solution at 37°. Histamine release was initiated by the simultaneous addition of antigen (40 μg/ml) and the concentrations of N^6-methyladenosine indicated. After 10 min at 37°, the reactions were terminated and histamine release was analyzed as described in Materials and Methods. The per cent histamine release in the control sample was 24.2 ± 0.4 (corrected for a spontaneous release of 2.6 per cent). Vertical bars represent ± S.E.M. The single asterisk (*) indicates significantly different from control ($P < 0.05$). The double asterisk (**) indicates significantly different from control ($P < 0.001$).

Table 2. Ability of theophylline to block potentiation of histamine release by 10^{-5} M N^6-methyladenosine*

Concentration of Theophylline (M)	% Potentiation by N^6-Methyladenosine (10^{-5} M)
0	22.8 ± 3.6 (P<0.01)†
10^{-6}	17.7 ± 1.0 (P<0.05)
10^{-5}	13.2 ± 3.5 (P<0.05)
10^{-4}	8.9 ± 5.5 (NS)

* Lung tissue from four sensitized guinea pigs was combined for use in this experiment. The chopped lung tissue was prewarmed in Tyrodes solution at 37° for 5 min prior to the addition of N^6-methyladenosine, theophylline or the combination of the two. After 5 min, the tissue was challenged with 40 μg/ml of egg albumin. Reactions were terminated and histamine release was analyzed as described in Materials and Methods. The percentage histamine release in this experiment was 26.9 per cent (corrected for a spontaneous release of 3.7 per cent).

† The values for the per cent potentiation of histamine release are the means ± S.E. of four determinations. The 'P' values indicate the level of significance of the release obtained in the presence of N^6-methyladenosine alone or N^6-methyladenosine and theophylline in relation to the release obtained with antigen alone. NS = not significant.

Table 3. Effects of adenosine and adenosine analogs on the potentiation of ionophore-induced histamine release from purified rat peritoneal mast cells*

Analog (100 μM)	Potentiation of histamine release (%)
Adenosine	151.6 ± 12.9 (P<0.05)*
N^6-Methyladenosine	95.6 ± 11.3 (P<0.01)
2-Methyladenosine	87.0 ± 19.6 (P<0.05)
2-Chloroadenosine	85.0 ± 5.7 (P<0.01)
N^6-Phenylisopropyladenosine	136.5 ± 13.1 (P<0.01)
5'-Deoxyadenosine	33.3 ± 2.0 (NS)
2'-Deoxyadenosine	26.0 ± 9.5 (NS)
2',5'-Dideoxyadenosine	20.5 ± 5.7 (NS)

* Purified cells (1×10^5 cells/ml) were prewarmed for 9 min at 37° prior to the addition of adenosine or the indicated adenosine analogs. After 1 min, the cells were challenged with 3×10^{-8} M ionophore A23187. Reactions were terminated and histamine release was quantitated as described in Materials and Methods. The per cent histamine release in this experiment was 14.3 ± 1.4 (corrected for a spontaneous release of 4.2 per cent).

† The values for the per cent potentiation of histamine release are the means ± S.E. of four determinations. The 'P' values indicate the level of significance of the difference in the release obtained in the presence of adenosine or analog in relation to the release obtained with antigen alone. NS = not significant.

et al. [17] might be due to the ability of inosine to inhibit cellular adenosine deaminase, thus producing a rise in endogenous adenosine levels.

Effects of adenosine and adenosine analogs in guinea pig lung adenylate cyclase. The data presented in Table 4 demonstrate that only the analogs with strong 'P' site activity, 9-β-D-arabinofuranosyladenine, 5'-deoxyadenosine, 2-deoxyadenosine and 2',5'-dideoxyadenosine, mimic the ability of adenosine to inhibit guinea pig lung adenylate cyclase in a dose-related fashion. The relative order of potency for inhibition by these analogs is 2',5'-dideoxyadenosine > 5'-deoxyadenosine ≫ 2'-deoxyadenosine ≅ 9-β-D-arabinofuranosyladenine > adenosine. This is similar to the order of potency observed for 'P' sites associated with other adenylate cyclase systems [24, 26]. It should be noted that the IC_{50} values reported in this table were obtained when the enzyme was assayed in the presence of 5 mM $MgCl_2$. As previously reported by Weinryb and Michel [25], the potency of inhibition of adenylate cyclase by adenosine and the other 'P' site analogs tested was extremely dependent upon the concentration of $MgCl_2$ used in the assay. Also, inhibitory potency can be influenced by the type of metal ion exposed to the system, by guanine nucleotides, and in some cases, by the presence of epinephrine or isoproterenol in the assay.*

* A. F. Welton and B. A. Simko, manuscript in preparation.

Table 4. Inhibition of guinea pig lung adenylate cyclase by adenosine and adenosine analogs*

Analog	IC_{50} (M)
Adenosine	6×10^{-4}
9-β-D-Arabinofuranosyladenine	1.8×10^{-4}
2′-Deoxyadenosine	1.6×10^{-4}
5′-Deoxyadenosine	4.8×10^{-5}
2′,5′-Dideoxyadenosine	2.8×10^{-5}
2-Chloroadenosine	$>10^{-3}$
N^6-Methyladenosine	NI†
2-Methyladenosine	NI
N^6-Phenylisopropyladenosine	NI
Formycin B	NI
Adenine	$>10^{-3}$

* The adenylate cyclase assay was performed as described in Materials and Methods.
† Not inhibitory.

Attempts to observe any type of 'R' site effect (inhibitory or activating) on guinea pig lung adenylate cyclase were unsuccessful. During such attempts, assay conditions were varied to include different types of metal ions, guanine nucleotides, and other enzyme activators such as β-agonists, histamine and prostaglandins. Also, assays were conducted in the presence of high concentrations of adenosine deaminase (up to 12 units/ml of enzyme assay) to purge the system of any endogenous adenosine which might be generated from substrate ATP by nucleotide-metabolizing enzymes present in the lung homogenate. This was done to try to eliminate the possibility that endogenous adenosine might bind with such high affinity to 'R' sites associated with lung adenylate cyclase and thereby mask the effects of exogenous 'R' site agonists, such as N^6-methyladenosine. Finally, attempts were made to assay the adenylate cyclase activity in guinea pig lung using 2′-deoxy-ATP as a substrate.* Utilizing this substrate, only 2′-deoxyadenosine should be formed by the nucleotide-degrading enzymes in the lung homogenate. Since 2′-deoxyadenosine would function only as a 'P' site agonist at the concentrations which might be present during enzymatic assay, this system allows the observation of 'R' site effects in the absence of endogenous 'R' site ligands. However, experiments with this system, too, did not reveal 'R' site effects on the lung adenylate cyclase.

DISCUSSION

The results of this study demonstrate that adenosine potentiates the antigen-induced release of histamine from the lung tissue of actively sensitized guinea pigs. This is similar to the previously observed effect of adenosine on mediator release systems associated with rat peritoneal mast cells [17], but differs from observations made with human basophils in which adenosine actually inhibits mediator release [36]. It is difficult to speculate on a reason for the differences observed in these secretory systems except to note that the systems must have some fundamental differences in their secretory mechanisms. The differential sensitivity of these systems to inhibitors of mediator release, such as disodium cromoglycate [37–39], would support such a conclusion. Alternatively, it is known that basophil preparations are contaminated with other cells which also respond to adenosine, such as platelets [15, 16] and lymphocytes [11, 12]. Thus, another explanation is that, in the presence of adenosine, these contaminating cells release regulatory molecules which alter the ability of adenosine to modulate histamine release from basophils.

The site through which adenosine interacts to potentiate mediator release from lung and purified mast cells was characterized in our studies as being an 'R' site by the criteria established by Londos and Wolff [27]; that is, only analogs with strong 'R' site activity, 2-methyladenosine, N^6-methyladenosine, 2-chloroadenosine and N^6-phenylisopropyladenosine, were found to mimic the potentiating capabilities of adenosine. The observation that theophylline blocks the potentiating ability of adenosine in the guinea pig lung system, which was also previously described by others for rat peritoneal cells [17], is consistent with the finding that adenosine is acting at such a site [27].

The role of adenosine in mediator release in lung and mast cells appears to be modulatory. Adenosine itself does not affect the spontaneous release of histamine; the presence of a release inducer such as antigen, ionophore A23187, concanavalin A, compound 40/80 or anti-IgE, is required in order to observe an effect with the nucleotide. Yet, the studies performed to date have not eliminated the possibility that adenosine may actually be required for release. Since enzymes such as ATP hydrolases and 5′-nucleotidase are present in the exterior of cells and since under normal release-inducing conditions

* The studies were performed at the suggestion of Dr. C. Londos, of the National Institute of Arthritis, Metabolism and Digestive Diseases, NIH, who has used such an assay system to observe 'R' sites in the liver adenylate cyclase system.

these enzymes could be metabolizing ATP and 5'-AMP to adenosine, there would always be endogenous adenosine present in the exterior of the cell to participate in the release reaction. The 'R' adenosine sites are believed to be receptors located on the cell exterior [27] and because these receptors appear to have very high affinities for adenosine ($\approx 10^{-7}$ M giving half-maximal effect in the mast cell [17]), adenosine concentrations in the range required to promote mediator release could always be present near the sites. If this is true, one cannot eliminate the possibility that adenosine is an absolute cofactor required in the release process. At present we are attempting to establish conditions under which to examine this question more thoroughly.

The potential for the continuous presence of endogenous adenosine in mediator release systems might also explain another observation we have made with regard to the ability of exogenous adenosine to potentiate mediator release in lung tissue and purified rat peritoneal mast cells. From a comparison of the data observed in Tables 1 and 3, it can be seen that much larger potentiations are observed in the purified mast cell system than in lung tissue. This may be due to the fact that in lung tissue the cells are quite concentrated and closely associated with one another. Hence, the endogenous adenosine levels present near exterior 'R' site receptors would most likely be higher than in the purified mast cell system where the cells are more dilute and where endogenous adenosine produced by the cells is much more free to diffuse away from the receptor sites. Alternatively, the less striking potentiation of histamine release in the lung might also reflect the possibility that adenosine is affecting more cell types in the lung than just the mast cell. As discussed previously for basophil preparations, adenosine may be causing the release in the lung of regulatory substances which alter the histamine release potentiating ability of adenosine.

The inhibitory effect of adenosine on guinea pig lung adenylate cyclase previously described by Weinryb and Michel [25] does not appear to be related to the ability of this nucleoside to potentiate mediator release from the lung; that is, our studies have demonstrated that inhibition appears to be modulated through a 'P' site for adenosine, not an 'R' site. These findings demonstrate the importance of comparing the characteristics of the adenosine receptors associated with a physiological process and with adenylate cyclase before assuming that this enzyme is involved in the mechanism by which the physiological process is modulated by the nucleoside. Due to the heterogeneity of cell types present in the lung, however, our studies cannot eliminate the possibility that adenosine does act through an 'R' site associated with a subpopulation of adenylate cyclase present in the lung in such small quantities that it cannot be observed by assaying a total lung homogenate. Future studies, therefore, will be directed at characterizing the effects of the nucleoside on the adenylate cyclase present in purified mast cells.

An obvious explanation for the lack of correlation between effects of adenosine on mediator release and in adenylate cyclase activity is that adenosine is not acting through this enzyme system at all. One possibility is that the potentiation phenomenon is due simply to increased cellular ATP levels. However, since 2-chloroadenosine and N^6-phenylisopropyladenosine are active in the potentiation process and these two nucleosides are not readily incorporated into nucleotide pools [40–42], this seems unlikely. Also, the observation that theophylline blocks the potentiation process argues against this explanation. It is possible, however, that other enzymes such as protein kinase [43, 44] or phosphodiesterase [6] are affected by adenosine. These possibilities will be more thoroughly examined in the future.

Thus, these studies have established a role for adenosine in regulating antigen-induced mediator release from lung tissue. In addition, the receptor through which this nucleoside acts to induce mediator release in lung tissue and purified peritoneal mast cells has been characterized as an 'R' adenosine receptor site. The mechanism by which this nucleoside acts through this receptor site to induce this effect is not known. And, it remains to be established whether adenosine plays simply a modulatory role in the release process or is absolutely required for release to occur.

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