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Effects of Adenosine on Histamine Release from Human Lung Fragments

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Abstract
The actions of adenosine on histamine release of human lung fragments were investigated. Histamine release was stimulated either with the calcium ionophore A 23187 or with concanavalin A. Adenosine and its analogue 5′-N-ethylcarboxamidoadenosine alone had no significant effect on basal release or on the release elicited by A 23187 or concanavalin A. However, in the presence of the adenosine receptor antagonist 8-[4-[[[[(2-aminoethyl)amino]-carbonyl] methyloxy]-phenyl]-1,3-dipropylaxanthine (XAC), which itself did not affect the release, adenosine increased the stimulated histamine release. On the other hand, in the presence of the nucleoside transport inhibitor S-(*p*-nitrobenzyl)-6-thioninosine (NBTI), adenosine caused a reduction in stimulated histamine release. NBTI itself caused a stimulation of release. Thus, a stimulatory effect of adenosine was seen in the presence of XAC, whereas an inhibitory effect was unmasked by NBTI. From these data it is concluded that adenosine exerts two opposing effects on histamine release in the human lung which neutralize each other: it inhibits release via a site antagonized by XAC, which presumably represents an A_2 adenosine receptor, and it stimulates release via a mechanism that is blocked by NBTI, suggesting that adenosine needs to reach the interior of cells to exert this effect. The slight stimulatory effect of NBTI alone demonstrates that trapping intracellularly formed adenosine inside mast cells leads to sufficient concentrations of adenosine to stimulate histamine release. These findings suggest an important bimodal role of adenosine in regulating histamine release in the human lung.

Introduction

Adenosine appears to play a role in the pathophysiology of bronchial asthma by modulating mast cell mediator secretion. There is also increasing evidence that during antigen provocation and hypoxia adenosine itself is released [1–3] and therefore should be considered as an additional mediator of allergic reactions.

Many of the actions of adenosine seem to be mediated by two types of cell surface receptors: the A_1 adenosine receptor which inhibits and the A_2 adenosine receptor which activates adenylate cyclase. Millimolar concentrations of adenosine may also act at an intracellular purine site with a resultant inhibition of adenylate cyclase [4, 5]. In studies with human basophils, adenosine elevates cyclic AMP via an A_2 adenosine receptor-mediated mechanism and in-

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hibits histamine release [6–8]. In contrast, adenosine potentiates mediator secretion from rat peritoneal mast cells [9, 10]. Several laboratories have recently reported that this stimulation could not be antagonized by adenosine receptor antagonists, such as theophylline. Two different hypotheses have been put forward to interpret this finding: some authors postulate the existence of a novel type of cell surface adenosine receptor that is not blocked by methylxanthines [11–13]. This proposal is supported by a modulation of the effects of adenosine by pertussis toxin and cholera toxin, known to affect G-protein function. Observations made in our laboratory, however, seem to argue against a cell surface action of adenosine to enhance mediator release. Because the nucleoside transport inhibitor S-(*p*-nitrobenzyl)-6-thioinosine (NBTI) abolished these effects of adenosine, we concluded that adenosine acts at an intracellular site [10]. Stimulation of this site appears to result in an enhanced sensitivity of the release process to calcium and, hence, results in enhanced release of mediators.

In addition to these effects on mast cell mediator release, adenosine and its analogue 5′-N-ethylcarboxamidoadenosine (NECA) activate adenylate cyclase and elevate intracellular cyclic AMP levels of intact rat peritoneal mast cells. This elevation of cyclic AMP levels is antagonized by theophylline and is thought to be mediated by A_2 adenosine receptors. Hence, an action of adenosine at two distinct sites in mast cells has been assumed [10].

However, recent studies show that mast cells and basophils from different species and even from different sites in the same species may differ in structure and function [14, 15]. In particular, the effect of adenosine on human lung mast cells is still controversial. Potentiation [16–18] as well as inhibition [16, 17, 19–21] of mediator secretion have been described. Methylxanthines antagonized both potentiation and inhibition in dispersed human lung mast cells [17], but antagonized only inhibition in human lung fragments [19]. The receptor subtype was identified as A_2 by the rank order of potency of adenosine and its synthetic analogues [17, 19]. However, recent studies suggested a distinct, unclassified adenosine receptor responsible for potentiation of histamine release [18, 22].

The present study was undertaken in order to see whether the dual control of mediator release from rat peritoneal mast cells – i.e. inhibition via A_2 receptors and stimulation via a presumably intracellular site [10, 23] – could also be detected in the human lung and could explain the contradictory effects described above. We chose to investigate this question using human lung fragments for three reasons: first we were interested in the net effect in the complex environment of the lung rather than in effects observable in isolated cell populations. Second, in contrast to isolated pulmonary mast cells, they contain the different mast cell subpopulation in their original composition, and third, lung fragments offer the advantage that cells are not altered by enzymatic treatment and long incubation periods in the absence of calcium known to decrease reactivity to secretagogues [24]. Although mast cells constitute only 2% of the lung tissue, they contain most of the tissue histamine stored in cytoplasmatic granules [25]. Thus histamine release of lung fragments represents the function of lung mast cells.

Materials and Methods

Materials

Adenosine was obtained from Boehringer, Mannheim, FRG, and concanavalin A from Serva, Heidelberg, FRG. The calcium ionophore A 23187, NBTI and histamine were purchased from Sigma, München, FRG. NECA was a gift from Byk Gulden, Konstanz, FRG, and the xanthine amine congener 8-[4-[[[[(2-aminoethyl)amino]-carbonyl]methyloxy]-phenyl] -1,3-dipropylxanthine (XAC) was donated by Dr. Entzeroth, Thomae, Biberach, FRG. All drugs were of analytical grade.

Preparation of Human Lung Tissue

Specimens of macroscopically normal areas of peripheral human lung were obtained at the time of surgery for carcinoma of the bronchus and were immediately immersed in Hepes-buffered saline (4 °C). Within 1 h, the tissue was transported on ice to the laboratory. All subsequent steps were carried out at 4 °C. The lung tissue was dissected free of pleura, large bronchi and blood vessels and chopped into 1 × 0.2 × 0.2 mm fragments using a McIllwain tissue chopper. Tissue fragments were then suspended in Hepes-buffered saline containing 137 m*M* NaCl, 2.7 m*M* KCl, 0.4 m*M* NaH_2PO_4, 10 m*M* Hepes, 5.5 m*M* glucose and bovine serum albumine 0.05%, pH 7.4 (adjusted with NaOH before use) and were centrifuged for 5 min at 120 *g*. This washing step was repeated three times, and the tissue fragments resuspended in incubation buffer which was washing buffer complemented with 1 m*M* $CaCl_2$ and 0.5 m*M* $MgCl_2$.

Histamine Release Experiments

The incubation was performed in duplicates at 37 °C with gentle mechanical agitation. Controls were fragments incubated in buffer alone. Approximately 40 mg lung tissue (wet weight) was preincubated for 10 min in 0.95 ml incubation buffer containing XAC or NBTI. NECA and adenosine were added 5 min before stimulation. Histamine release was stimulated by adding 50 µl incubation buffer with either calcium ionophore A 23187 (1 µ*M* final concentration unless indicated otherwise) or concanavalin A (1 mg/ml). The histamine release was terminated by chilling the samples to 4 °C and centrifugation at 300 *g* for 10 min.

Extraction and Fluorometric Assay of Histamine

For extractions of histamine from supernatant and pellet and the fluorometric assay the method of Shore et al. [26] was used. Protein

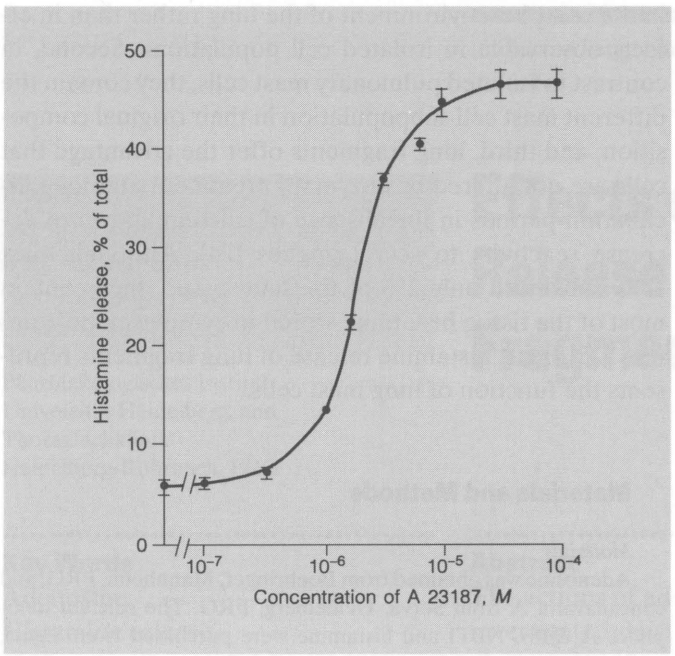

Fig. 1. Effect of the calcium ionophore A 23187 on histamine release from human lung fragments at 90 min of incubation. Values are means ± SEM of five separate experiments with duplicate samples.

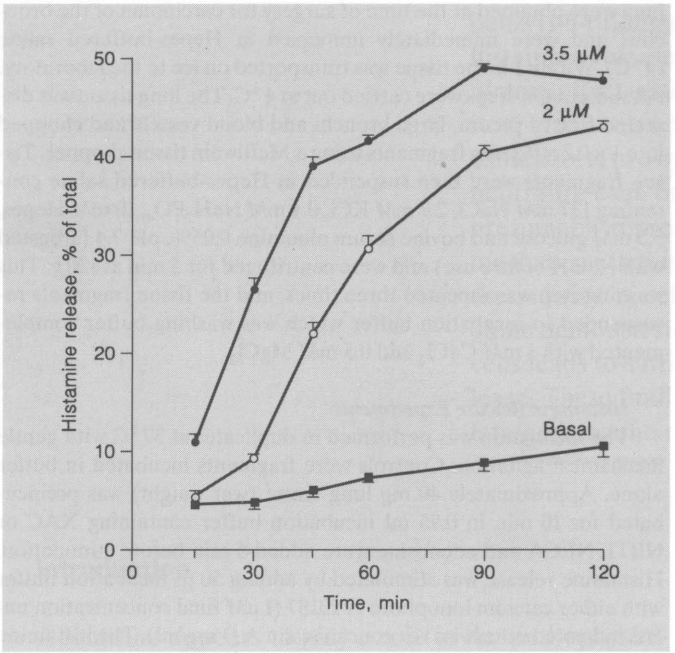

Fig. 2. Time course of the calcium ionophore A 23187-induced histamine release. Experiments were performed with 2 and 3.5 μ*M* final concentrations of the calcium ionophore or without stimulation (basal release). Values are means ± SEM of four separate experiments with duplicate samples.

from the tissue pellet and the supernatant was removed by perchloric acid precipitation. Histamine was then extracted into n-butanol from the alkalinized salt-saturated sample. The histamine was recovered in an aqueous solution of 0.1 *N* HCl by adding heptane. This solution was then used for the condensation of histamine with opthalaldehyde modified by the citric acid stop [27] and the condensation reaction at 0 °C [28]. Histamine release was expressed as a percentage of the total histamine content (tissue plus medium). The experiments were repeated 4–5 times, and for every separate experiment a tissue preparation of an individual patient was used. The total histamine content of the human lung tissue ranged from 7 to 25 μg/g lung tissue (9.8 ± 0.1 g mean ± SEM, n = 79) and thus was in good agreement with results obtained in other studies [27, 29, 30].

Statistics

Statistical significances of nucleoside-related effects were analyzed by comparing control and nucleoside-treated tissue fragments using Student's t test for paired data. Values were considered significant at $p < 0.05$.

Results

Stimulation of Histamine Release by A 23187 and Concanavalin A

Human lung fragments incubated for 90 min with the calcium ionophore A 23187 showed a concentration-dependent increase in histamine release with an EC_{50} value of 1.7 μ*M*. Maximal stimulation occurred at a concentration of 30 μ*M*, which induced a histamine release of 46.4 ± 1.4% (mean ± SEM) of total tissue content (fig. 1). In all experiments, spontaneous histamine secretion was less than 10% during 90 min of incubation and maximally 12.4% at 120 min.

The rate of histamine release from human lung fragments depended on the degree of stimulation. The time for histamine release to reach 50% of its eventual maximum ($t_{1/2}$) was approximately 45 min at a concentration of 2 μ*M* A 23187 and the release was complete by 120 min. With 3.5 μ*M* of the calcium ionophore, $t_{1/2}$ was 30 min, and maximal histamine secretion was reached at 90 min (fig. 2).

Concanavalin A also induced a time- and dose-dependent release of histamine. Compared with the stimulation by the calcium ionophore, the release was more rapid, but the maximal release was less. The highest concentration used was 1 mg/ml concanavalin A resulting in a histamine release (mean ± SEM) of 15 ± 0.8% of total histamine content at 30 min of incubation with a spontaneous release of 7.5 ± 0.1% (mean ± SEM, $p < 0.05$). $T_{1/2}$ was approximately 8 min (fig. 3).

Effects of Adenosine and NECA on Calcium Ionophore-Induced Histamine Release

Approximately 30% of maximal histamine release was achieved by stimulation of human lung fragments with 1 μ*M* calcium ionophore. Adenosine and its analogue NECA had no effect on basal (6.4±1.2% without, 7.2±1.3% with 100 μ*M* adenosine and 6.6±1.3% with 10 μ*M* NECA, mean ±SEM of three independent experiments after a 90-min incubation period) or A 23187-induced histamine release (fig. 4, 5). To determine whether extracellular adenosine receptors are involved, the adenosine receptor antagonist XAC was used [31]. XAC (1 μ*M*), which is one of the most effective A_2-antagonists at the moment, had no influence on basal histamine release (6.4±1.2% without and 6.6±1.1% with XAC, mean ±SEM of three independent experiments after 90 min of incubation) or on release stimulated by A 23187 (fig. 4). However, in the presence of XAC, 100 μ*M* adenosine significantly enhanced stimulated histamine release (fig. 4). The stimulation-evoked release was enhanced by almost 40%. A similar enhancement was observed with 10 μ*M* of the metabolically stable adenosine analogue NECA (fig. 5). The nucleoside transport inhibitor NBTI (1 μ*M*) had no effect on histamine release in unstimulated human lung fragments (basal histamine release: 6.4±1.2%, with NBTI: 7.2±1.5%, mean ±SEM of three independent experiments after 90 min of incubation). However, in the presence of 1 μ*M* NBTI, the calcium ionophore-induced histamine release was significantly enhanced (fig. 4). In the presence of 1 μ*M* NBTI, adenosine inhibited histamine release, whereas adenosine alone had no influence on histamine release (fig. 4). The experiments involving NBTI were not done with NECA, because NECA can diffuse across the cell membrane so that blockade of the nucleoside transporter would have little effect on its distribution.

Effects of Adenosine on Concanavalin A-Induced Histamine Release

To confirm that adenosine not only modulates calcium ionophore-induced histamine release, we also challenged human lung fragments by bridging IgE molecules with concanavalin A (1 mg/ml). The findings were similar to those obtained with the calcium ionophore. Adenosine (100 μ*M*) alone has no effect on the histamine release of activated human lung fragments (fig. 6). However, in the presence of XAC (1 μ*M*), which had no effect on histamine release, adenosine potentiated the stimulated histamine release. In the presence of 1 μ*M* NBTI, adenosine inhibited histamine release (fig. 6). Stimulation and inhibition by adenosine were more rapid when lung fragments were

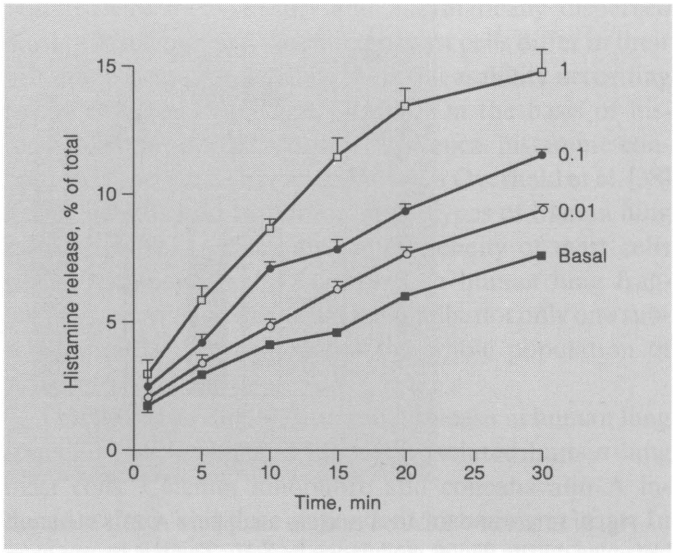

Fig. 3. Time course of concanavalin A-induced histamine release. Experiments were performed with 0.01, 0.1 and 1 mg/ml final concentrations of concanavalin A or without stimulation (basal release). Values are means ± SEM of four separate experiments with duplicate samples.

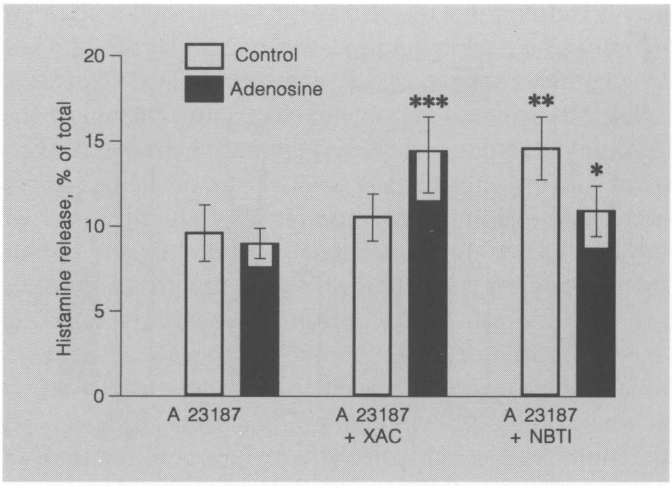

Fig. 4. Effects of adenosine on calcium ionophore A 23187-induced histamine release and its modulation by XAC and NBTI. The release was measured after stimulation with 1 μ*M* calcium ionophore A 23187 and in the presence of 1 μ*M* XAC or 1 μ*M* NBTI. Human lung fragments were incubated for 90 min with and without (control) 100 μ*M* adenosine. The values show A 23187-induced histamine release corrected for basal histamine release, that was 7.5±1.0% of total histamine content (mean ±SEM). Statistically significant levels of potentiation or inhibition compared to control (* $p < 0.05$) and compared to A 23187-stimulated samples (** $p < 0.05$) are given. Values are means ±SEM of four separate experiments with duplicate samples.

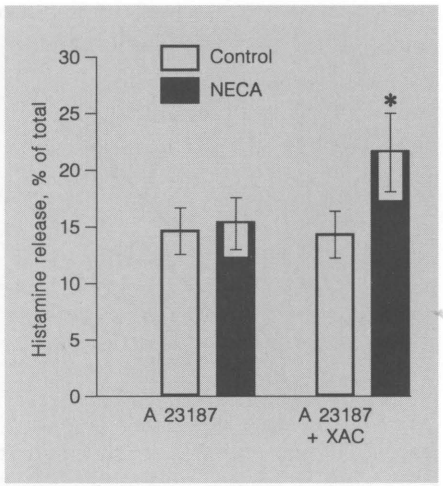

Fig. 5. Effect of NECA on calcium ionophore A 23187-induced histamine release and its modulation by XAC. The release was measured after stimulation with 1 μ*M* calcium ionophore A 23187 and in the presence of 1 μ*M* XAC. Human lung fragments were incubated for 90 min with and without (control) 10 μ*M* NECA. The values show A 23187-induced histamine release corrected for basal histamine release, that was 9.2 ± 0.7% of total histamine content (mean ± SEM). Statistically significant levels of potentiation or inhibition compared to control (* $p < 0.05$) are given. Values are means ± SEM of five separate experiments with duplicate samples.

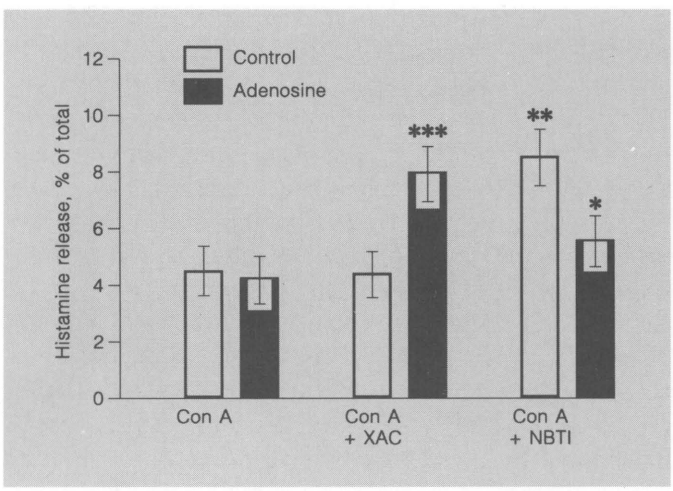

Fig. 6. Effect of adenosine on concanavalin A-induced histamine release and its modulation by XAC and NBTI. The release was measured after stimulation with 1 mg/ml concanavalin A (con A) and in the presence of 1 μ*M* XAC or 1 μ*M* NBTI. Human lung fragments were incubated for 60 min with and without (control) 100 μ*M* adenosine. The values show concanavalin A-induced histamine release corrected for basal histamine release, that was 6.4 ± 1.1% of total histamine content (mean ± SEM). Statistically significant levels of potentiation or inhibition compared to control (* $p < 0.05$) and compared to concanavalin A-stimulated samples (** $p < 0.05$) are given. Values are means ± SEM of four separate experiments with duplicate samples.

challenged with concanavalin A. A significant effect was already seen at 15 min of incubation (data not shown). Thus, adenosine was found to modulate histamine release from human lung tissue in a similar manner when release was stimulated by either A 23187 or concanavalin A. It inhibited release in the presence of NBTI and it stimulated release in the presence of XAC.

Discussion

Histamine release of human lung fragments was modulated by adenosine and its analogue NECA via two different mechanisms. NECA and adenosine alone did not influence mediator secretion activated by the calcium ionophore A 23187 or concanavalin A. In the presence of the adenosine receptor antagonist XAC, adenosine and NECA markedly increased calcium ionophore- and concanavalin A-induced histamine release. Because this potentiation was observed after blockage of cell surface adenosine receptors, it is concluded that it is not mediated via a classical adenosine receptor. To determine the site of action where adenosine stimulates histamine release, the nucleoside transport inhibitor NBTI was used. The movement of nucleoside molecules across the plasma membrane is a facilitated, bidirectional process mediated by specific nucleoside transporters [32]. Thus influx and efflux of adenosine can be prevented by blockade of this transporter [32]. Abolishment of this transmembrane exchange by NBTI can increase intracellular adenosine concentrations, trapping intracellularly generated adenosine inside the cell. Incubation of NBTI with activated human lung fragments enhanced histamine release, in agreement with the hypothesis [10, 23] that the stimulatory effect of adenosine is mediated by an intracellular mechanism. When NBTI blocked the transport of adenosine across the plasma membrane, extracellular exogenous adenosine inhibited the NBTI-induced histamine release. Under these conditions, exogenous adenosine could not enter the cell, suggesting that adenosine inhibits mediator secretion via a cell surface adenosine receptor. According to the kinetics of the histamine release, both effects of adenosine were more rapid after stimulation with concanavalin A compared to the calcium ionophore.

Since in human lung mast cells the adenosine receptor appears to be of the A_2 subtype [17, 19], which mediates a stimulation of adenylate cyclase, our results concur with studies that show an association of elevated cyclic AMP levels with inhibition of mediator release in human basophils and lung mast cells [33].

Different ways of activating mast cells by concanavalin A or the calcium ionophore A 23187 [34] caused a similar enhancement in histamine release by adenosine. This suggests that adenosine acts on a step beyond receptor activation. From our results, it cannot be excluded that adenosine facilitated the transport of calcium into the cell. However, studies with rat peritoneal mast cells [23, 35] showed an unchanged intracellular calcium concentration and suggested therefore as a mechanism an increase in the calcium sensitivity of the release process. Concurring with these results in rat peritoneal mast cells [23], we found that trapping of adenosine inside the cell with the nucleoside transport inhibitor NBTI caused enhancement of histamine release. Therefore, concentrations of endogenously generated adenosine seem to be sufficient to potentiate the release in human lung fragments.

Adenosine, as an inflammatory mediator itself, is generated and possibly released after stimulation of mast cells [36]. Because of the bidirectional transport of nucleosides, high levels of adenosine in the extracellular space correspond to elevated levels of intracellular adenosine [32]. Thus, adenosine appears to act through a positive feedback mechanism. By increasing the sensitivity of the release process to free intracellular calcium, a potentiation of mediator release is achieved [35].

Others have suggested that the enhancing effects of adenosine on mast cell mediator release might be caused by activation of an atypical adenosine receptor. To reconcile our data with this hypothesis, we would have to assume that NBTI also acts as a full agonist at this receptor. While we cannot rule out this possibility, we feel that our interpretation is more likely to be true, because NBTI has been proved to be a very specific and potent nucleoside transport inhibitor in all tissues and cells tested.

Ali et al. [13] recently reported the activation of phospholipase C in a rat tumor mast cell (RBL-2H3) by adenosine and suggested that the resultant stimulation of protein kinase C might be the basis of the enhancement in mediator release. In accordance with our results, the authors reported that methylxanthines did not block these effects of adenosine. However, since the effects of adenosine were abrogated by pertussis toxin as well as cholera toxin, it was assumed that adenosine acted on an atypical G-protein-coupled receptor. No experiments using adenosine uptake blockers were done, and thus further studies are necessary to establish the relationship between these data and ours. Clearly, effects mediated via intracellular G proteins would appear to be a possibility.

In this study, human lung fragments were chosen to investigate the effects of adenosine on histamine release. Studies with mechanically and enzymatically dispersed mast cells have shown that human mast cells differ in their histamine content as well as their releasability according to the isolation procedure [21, 37]. On the basis of histochemical properties, density difference, histamine content and functional characteristics, van Overheld et al. [38] have characterized two different subtypes of human lung mast cells. This reflects the heterogeneity of mast cells within the lung tissue. Therefore, in human lung fragments in contrast to dispersed mast cells, not only one subpopulation is investigated but the whole population of mast cells.

The characteristics of histamine release in human lung fragments were similar to those in isolated human lung mast cells. Calcium ionophore and concanavalin A induced a time- and dose-dependent histamine release. In agreement with previous reports [18, 29, 39, 40], histamine release induced by the calcium ionophore was more pronounced than that by concanavalin A reaching maximally 46.4 and 15% of total histamine content, respectively. Compared to isolated human lung mast cells, histamine release in the lung fragments was slower. This is probably due to impaired penetration of the stimulating agent and delayed diffusion of the mediator. As in previous studies, histamine release induced by concanavalin A was more rapid than that caused by the calcium ionophore [29], and the rate of release depended upon the degree of stimulation [16]. These results show that histamine release in tissue fragments reflects the function of the lung mast cells.

In summary, histamine release from human lung fragments appears to be regulated by adenosine in a dual manner: potentiation and inhibition of histamine release, resulting overall in an unchanged mediator secretion. Concentrations of adenosine achieved by trapping endogenously generated adenosine inside the cell with the nucleoside transport inhibitor NBTI were sufficient to potentiate histamine release. These data suggest that endogenous adenosine is capable to affect histamine release in the human lung. Since adenosine can either inhibit or stimulate histamine release, it appears to be a unique regulator of this process.

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