

Purine derivatives in the study of allergic inflammation in respiratory diseases

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Adenosine, a naturally occurring purine nucleoside, elicits dose-related bronchoconstriction in asthmatic subjects when administered by inhalation and it is recovered in increased amounts from the bronchial lavage fluid of subjects with active asthma when compared to normal controls. Although the mechanism by which adenosine mediates bronchoconstriction in asthmatic subjects is not clear, recent data indicate an important role for mast cell mediator release. We have recently shown that local airway challenge with adenosine in subjects with asthma and rhinitis provokes an increase in the levels of PGD₂, histamine and tryptase. However, airway responsiveness and atopic status are the most important determinants of adenosine-induced responses, regardless of any increases in mast cell mediators in airway fluids. New discoveries suggest that the airway response to adenosine may be an index of mast cell priming and therefore may provide a useful tool to further explore the inflammatory processes in allergic asthma and rhinitis. Therefore adenosine provocation may gain increasing acceptance as an additional measure of disease activity in asthma and rhinitis.

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A large number of chemical entities have been described as candidate mediators for asthma and rhinitis. Traditionally, these have been divided into "preformed mediators" such as histamine, and "newly-generated mediators", lipid-derived substances including prostaglandins and leukotrienes which are synthesised *de novo* on appropriate cell stimulation. Adenosine is a naturally occurring purine nucleoside that serves an autocoid function in a large number of physiologic systems, and may be considered as a secondary product of the inflammatory response (1).

Evidence that adenosine release may be increased under conditions which prevail in areas of inflammation and in the asthmatic lung has been recently obtained from studies in subjects with asthma and chronic bronchitis which have demonstrated increased amounts of adenosine in the bronchial lavage fluid (2). Once generated, adenosine elicits bronchoconstriction in asthmatic subjects (3) and nasal symptoms mimicking rhinitis in subjects with stable allergic rhinitis (4). Adenosine promotes most of its effector functions by stimulating specific cell surface receptors leading to either a decrease (A₁ receptor) or an increase (A₂ receptor) in cellular levels of cyclic 3', 5'-AMP

(cyclic AMP) (5). A₁ and A₂ receptors are defined by a unique agonist potency series of adenosine analogues to inhibit or stimulate adenylate cyclase activity, A₁ receptors being stimulated by L-N⁶-phenylisopropyladenosine (L-PIA) > adenosine > 5'-N-ethylcarboxamidoadenosine (NECA) whilst the A₂ receptors have a potency series of NECA > adenosine > L-PIA (6).

In recent years there has been a tendency for less importance to be attached to purine derivatives as mediators of allergic inflammation. However, new discoveries have now led to a resurgence of interest in its role as a unique diagnostic marker in allergic diseases.

The effector function of adenosine in the airways

On guinea pig tracheal smooth muscle preparations, adenosine and related analogues with preferential activity at A₂-receptors elicited a transient contraction followed by a more powerful and sustained relaxation (7, 8). Subsequent relaxation of an established contraction occurs through an increase in cellular levels of cyclic AMP. On the basis of these animal findings, it seemed reasonable

to propose a bronchodilator action for adenosine. However, investigations for its *in vivo* effects on human airways proved to the contrary.

Whilst inhalation of adenosine aerosols was shown to have no detectable effect on airway calibre in normal subjects, when administered to patients with asthma it resulted in a concentration-related bronchoconstriction rather than the expected bronchodilation (3). A degree of selectivity for the adenine moiety was suggested by the finding that the purine nucleoside guanosine, when inhaled over the same concentration range as adenosine, had no measurable effect on asthmatic airways (3). In addition, inhalation of the adenine nucleotides AMP and adenosine 5'-diphosphate (ADP) in equivalent molar concentrations as adenosine, produced almost identical effects on the airways (9). Since both nucleotides are dephosphorylated to yield adenosine, it is likely that the airway effects of AMP and ADP are mediated following their hydrolysis to the purine nucleoside (10). Further evidence that adenosine-induced bronchoconstriction is due to a specific stimulation of the airway purinoceptors is provided by the demonstration that theophylline, a xanthine derivative that at therapeutic concentrations behaves as an adenosine receptor antagonist (11), had a significant protective effect against inhaled adenosine and AMP when compared to any effects of this drug to functionally antagonise histamine-induced bronchoconstriction (12–14). The importance of theophylline interactions with adenosine receptors was recently highlighted by demonstrating rebound hyperresponsiveness to inhaled adenosine on withdrawal of chronic theophylline therapy (15). One plausible explanation of this is an upregulation of adenosine receptors. Any drug that inhibits cellular uptake of adenosine will increase its extracellular concentrations and would be expected to augment its bronchoconstrictor response. One such drug is dipyridamole. When administered to asthmatic patients by intravenous infusion (16) or by inhalation (17), dipyridamole enhances the bronchoconstrictor response to adenosine, thus suggesting the importance of a specific cell surface interaction between the nucleoside and effector cells.

Mechanisms of adenosine-induced bronchoconstriction

Despite intensive research over the past decade, the mechanism by which adenosine mediates bronchoconstriction and its importance as a mediator in clinical asthma remain to be investigated. Although it has not been possible to administer many of the adenosine antagonists to humans for toxicological reasons, alternative pharmacological

approaches have suggested that *in vivo* it is unlikely that adenosine acts directly on smooth muscle cells, but indirectly through activation of specific receptors on intermediary inflammatory cells such as mast cells or on afferent nerve endings.

That mast cell derived mediators are involved in the adenosine response is clearly indicated by *in vitro* studies which have shown that adenosine and synthetic analogues active at A₂ receptors markedly enhance histamine release (18) and prostanoïd generation (19) elicited from immunologically stimulated human lung mast cells. These findings align with the observation in clinical studies that pre-medication with antihistamines (20, 21) and potent inhibitors of cyclooxygenase (22–24) inhibit the bronchoconstrictor response to AMP inhalation in asthmatic subjects.

Additional support for mast cell involvement in adenosine-induced bronchoconstriction comes from the finding that sodium cromoglycate and nedocromil sodium, both considered inflammatory cell “stabilizers” (25), are highly effective in attenuating the airway response to adenosine and AMP challenge in asthmatic subjects (26, 27, 28), although one recent study has shown that sodium cromoglycate competes with NECA for binding sites on plasma membranes obtained from normal human lung suggesting that cromones may also act by adenosine receptor inhibition (29).

More direct evidence that preformed and newly generated mediators released from airway mast cells may indeed play a role in adenosine-induced responses comes from a series of studies in which a significant rise in plasma levels of histamine (30), neutrophil chemotactic factor – NCF (31) and thromboxane B₂ (32) was reported after adenosine challenge. Moreover, it has been recently reported that asthmatic airways respond to endobronchial challenge with adenosine with an immediate rise in BAL fluid levels of PGD₂, histamine and tryptase when compared to endobronchial sham challenge with saline (Fig. 1) (33). Preliminary studies with a model of nasal provocation with AMP indicate that this purine nucleotide can mimic many of the symptoms of rhinitis in a dose-dependent fashion and that the potent and specific H₁ receptor antagonist, cetirizine, abolished these symptoms in all the subjects studied (4). In addition, nasal challenge with AMP induced an immediate rise in histamine levels in the nasal lavage fluid (Fig. 2) (34). Taken together these findings support the view that mediator release from activated mast cells is also likely to take place in the human airways *in vivo* following adenosine exposure.

Enhancement of mast cell mediator release may not be the only mechanism accounting for the bronchoconstriction provoked by inhaled purines.

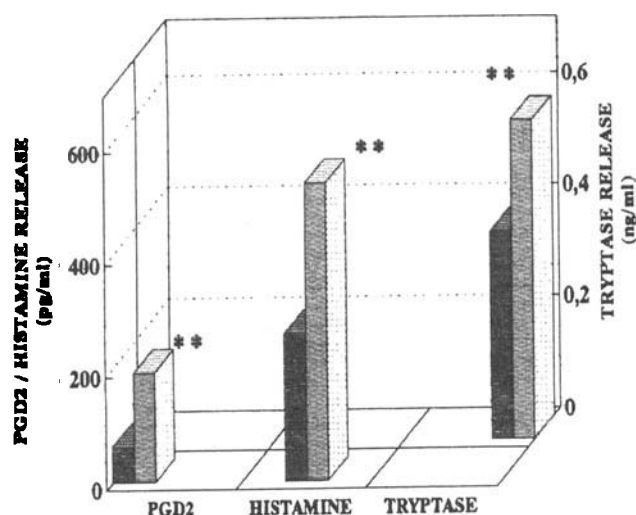


Fig. 1. PGD2, histamine and tryptase levels in the bronchoalveolar lavage fluid after local challenge with saline (black bars) and adenosine 5'-monophosphate (grey bars) obtained from asthmatic subjects. **significant difference at $P < 0.01$ vs saline.

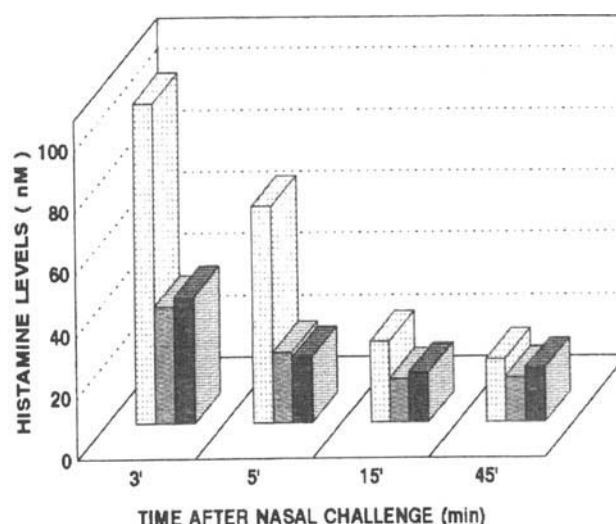


Fig. 2. Effect of adenosine 5'-monophosphate – AMP (dotted bars), guanosine 5'-monophosphate – GMP (grey bars), and saline (black bars) on nasal lavage histamine levels at different time-points.

Evidence is accumulating that local neural reflexes may also contribute to the contractile airway response to these autacoids in asthma. In isolated rabbit bronchial smooth muscle, adenosine potentiates the constrictor response to transmural nerve stimulation (35) and in inbred rats, the changes in airway response provoked by intravenous adenosine have been shown to have an atropine-sensitive component (36).

Despite initial negative results (37), there is some evidence that cholinergic neural pathways may also contribute to the airway effects of adenosine in subjects with stable asthma (38, 39). It is also possible that at least some of the reported protection afforded against the airways effect of adenosine and AMP by sodium cromoglycate and nedocromil sodium (26–28) is due to the ability of these drugs to inhibit peptidergic neural reflexes in addition to their effect on mast cells. Similarly, loop diuretics administered by inhalation may protect asthmatic airways against the immediate bronchoconstrictor response produced by AMP, possibly by modulating neural functions (40, 41). It has been shown that the 2-chloroadenosine induced bronchoconstriction in guinea pigs *in vivo* may well have a capsaicin-sensitive component (42), thus indicating that release of contractile neuropeptides from sensory nerve endings might be of some importance in mediating the airway effects of purine derivatives. In support of this view, it has been demonstrated that AMP shows some degree of cross-tachyphylaxis with bradykinin in asthmatic subjects (43).

Role of adenosine in airway inflammation

As there are no highly selective and specific adenosine antagonists that have acceptance for use in humans it is premature to implicate purines in the pathogenesis of asthma and rhinitis. Thus, at present their role is inconclusive but substantial evidence has accumulated in recent years to suggest their involvement in disease pathogenesis.

Adenosine is increased in inflammatory conditions of the airways. High concentrations of adenosine are measured in the BAL fluid of subjects with asthma and chronic bronchitis compared to normal controls (2). Studies in allergic asthmatic subjects have demonstrated enhanced levels of plasma adenosine within minutes of bronchial challenge with allergen (9). Moreover, adenosine levels have been reported to be significantly elevated in lung lavage fluid from allergic rabbit after antigen challenge (44). Taken together, these findings indicate that mechanisms for the production of adenosine are active during allergic disorders. However, the cellular source of adenosine in the airway fluids is not known.

All cells contain adenosine and adenine nucleotides. Adenosine release has been demonstrated to occur from rat peritoneal mast cells upon antigen challenge (45) and mast cells may have been a source of adenosine released into airway fluid since adenosine concentrations tended to be higher in those asthmatic subjects who also had high histamine concentrations (2). Besides mast cells, neutrophils and platelets are also found in increased numbers in the bronchial biopsies of asthmatics challenged with specific antigen (46). Platelets may

be another potential source of adenosine. Platelet activation elicits release of ADP, which could be then converted to adenosine. Similarly, neutrophils release 5'-AMP which is then converted to adenosine by a specific ecto-5'-nucleotidase (47). Epithelial injury is another prominent feature of asthma (48, 49). It is possible that adenosine may be derived from adenine nucleotides, which are released from damaged airway cells. Indeed, adenine nucleotides are present in large quantities in the cytoplasm of these cells and then converted to adenosine by the action of ectonucleotidase enzymes on the plasma membranes of lung cells (50).

Once generated, adenosine may promote a large variety of effector functions in the airways. Aside from its potential role as a mediator of bronchoconstriction and nasal symptoms, adenosine may also function as a novel paracrine mediator that contributes to various aspects of the inflammatory response. Asthma and rhinitis are currently considered to be inflammatory diseases associated with the pathological findings of cellular infiltration and oedema of mucosa. In this respect adenosine may have some important actions. Adenosine causes plasma exudation and increased bronchial blood flow (51). The ensuing presence of plasma proteins in the interstitium produces fluid efflux via osmosis and hence oedema. Adenosine may modulate inflammation by promoting neutrophil chemotaxis (52), and by enhancing histamine release from immunologically activated human lung mast cells and circulating basophils (19, 53).

It is now recognized that mast cells can also participate in more persistent and even chronic inflammatory responses by releasing multifunctional cytokines (54). Purified mast cells from normal human lung have been shown to release IL-4 (55). Furthermore, immunoreactivity for IL-3, IL-4 IL-5, IL-6, and GM-CSF has been localised to mast cells in bronchial biopsies (55-57). Finally, IL-8, a cytokine known to be a potent chemo-attractant for both neutrophils and eosinophils, is also produced by human lung mast cells (58). Taken together these findings suggest that another possible way by which adenosine could lead to the development of persistent airway inflammatory responses is through the release of mast cell derived cytokines (59).

Adenosine airway responsiveness and clinical correlates

Inflammation is a cardinal feature of asthma (60), the clinical expression of which appears to be airway hyperresponsiveness, the exaggerated bronchoconstrictor response to various stimuli

(61). The extent of this susceptibility can be assessed in the laboratory by bronchoprovocation tests with inhalation of histamine or methacholine (62). However, although asthma is linked with inflammation, the clinical and diagnostic relevance of airway responsiveness as currently defined is unclear. Indeed these bronchoprovocation tests are not suitable for confirming a diagnosis of asthma as they will not exclude chronic obstructive pulmonary disease or other respiratory disorders. The relatively poor specificity for asthma of histamine or methacholine challenge indicates that more appropriate laboratory tests need to be established. Assessment of airway hyperresponsiveness with indirect bronchoconstrictor stimuli, such as adenosine, may fill this gap.

Cushley et al. (3) were the first to demonstrate that adenosine provokes bronchoconstriction of rapid onset when inhaled by asthmatics but not normal subjects. Confirmation of these findings have been recently obtained *in vitro* where bronchi from asthmatic subjects were more sensitive to adenosine than those obtained from non-asthmatic controls (63). In this regard, adenosine may be unique since *in vitro* hyperresponsiveness was not demonstrated with other mediators such as histamine and leukotriene C₄ (63). In man, the airways response to inhaled adenosine is not accompanied by a late phase of bronchoconstriction or an increase in non specific bronchial responsiveness (64). Responsiveness of the airways to inhaled adenine derivatives correlates only weakly with more direct indices of airway responsiveness measured with such agonists as histamine (9) and methacholine (37).

More recently, Phillips et al. (30) have shown that atopic subjects, when compared to non atopic controls, are relatively more responsive to inhaled adenosine than they are to methacholine, thus suggesting that the airway response to these purines may be an index of mast cell priming. In this context, it is of interest that adenosine potentiates the release of inflammatory mediators when human mast cells are immunologically primed *in vitro* (18, 19). Moreover, nasal challenge with AMP exposure elicits nasal symptoms and an immediate rise in histamine levels in the nasal lavage fluid with the highest rise in atopic compared to non-atopic volunteers (Fig. 3) (34). This indicates that symptoms and histamine release might be better related to the atopic status. Thus, airway responsiveness and atopic status are the most important determinants of adenosine-induced responses, regardless of any increases in mast cell mediators in airway fluids.

In atopic asthmatics, there is strong evidence that mast cells recovered by BAL are increased in

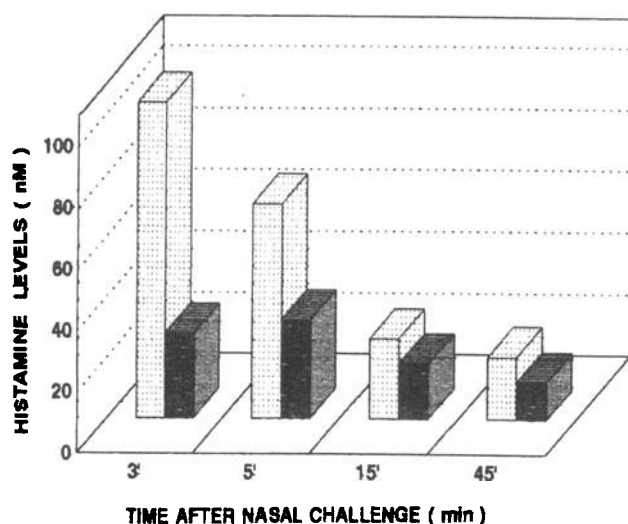


Fig. 3. Effect of adenosine 5'-monophosphate on histamine recovery in nasal lavage from atopic (dotted bars) and non-atopic (black bars) subjects at different time-points.

number (65–67) and that these cells exhibit increased spontaneous histamine and tryptase release when compared with mast cells from control subjects (66, 68). The capacity of adenosine to augment mediator release from mast cell in the presence of a “low-level” second stimulus, which may be immunologic or nonimmunologic, raises the possibility that this nucleoside elicits mediator release in asthmatic human airways by interacting with cytokine-“primed” mast cells on the surface of inflamed airways. Thus, purine-induced bronchoconstriction in asthmatics and normal atopics may well depend on the state of airway mast cell priming and, indeed, may be used as an *in vivo* test for this.

Indeed, nonsmoking adults with COPD were significantly less responsive to inhaled adenosine than nonsmoking asthmatics, whereas the sensitivity to methacholine was similar in both groups (69). In children, bronchoprovocation tests with inhaled adenosine discriminates asthma from pediatric COPD with a sensitivity and specificity of 85–90%, in contrast to methacholine challenge which fails to distinguish between the two (70). Taken together these findings indicate that adenosine challenge may be a very useful tool in the differential diagnosis of asthma and COPD in patients of all ages in whom the diagnosis is clinically uncertain.

Final considerations

There is abundant evidence that responses produced by adenosine are not a mere reflection of nonspecific airway hyperresponsiveness but they involve a selective interaction with activated

inflammatory cells in diseased airways and may be related to atopy. Therefore airway response to adenosine may provide a useful tool to explore further the inflammatory and immunologic processes in allergic-dependent disorders.

The time is ripe for a rigorous and critical evaluation of adenosine and related purine nucleotides as novel diagnostic markers in allergic diseases. Further studies are warranted to define the value of this unique stimulus in following disease activity and response to therapy.

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