British Journal of Pharmacology (2008) 153, S446–S456
 0007–1188/08 $30.00
www.brjpharmacol.org

REVIEW

LifeSciences2007

Adenosine receptors and asthma

RA Brown, D Spina and CP Page

King's College London, Sackler Institute of Pulmonary Pharmacology, Division of Biomedical and Health Sciences, London, UK

The accumulation of evidence implicating a role for adenosine in the pathogenesis of asthma has led to investigations into all adenosine receptor subtypes as potential therapeutic targets for the treatment of asthma. Selective A_1 receptor antagonists are currently in preclinical development since adenosine has been shown experimentally to mediate various features of asthma through this receptor such as bronchoconstriction, mucus secretion and inflammation. The A_{2A} receptor is expressed on most inflammatory cells implicated in asthma, and as A_{2A} stimulation activates adenylate cyclase and consequently elevates cAMP, selective A_{2A} receptor agonists have now reached clinical development. However, initial reports concerning their efficacy are inconclusive. A_{2B} receptor antagonists are also under investigation based on the rationale that inhibiting the effects of adenosine on mast cells would be beneficial, in addition to other reported pro-inflammatory effects mediated by the A_{2B} receptor on cells such as airway smooth muscle, epithelial cells and fibroblasts. Whilst the effects in pre-clinical models are promising, their efficacy in the clinical setting has also yet to be reported. Finally, adenosine A_3 receptor stimulation has been demonstrated to mediate inhibitory effects on eosinophils since it also elevates cAMP. However, some experimental reports suggest that A_3 antagonists mediate anti-inflammatory effects, thus the rationale for A_3 receptor ligands as therapeutic agents remains to be determined. In conclusion, establishing the precise role of adenosine in the pathogenesis of asthma and developing appropriate subtype selective agonists/antagonists represents an exciting opportunity for the development of novel therapeutics for the treatment of asthma.
British Journal of Pharmacology (2008) **153,** S446–S456; doi:10.1038/bjp.2008.22

Keywords: adenosine receptor; inflammation; asthma; mast cells; airway smooth muscle

Abbreviations: COPD, chronic obstructive pulmonary disease; HMC, human mastocytoma; IL, interleukin; PC, provocative concentration

Introduction

This article provides an introduction to the role of adenosine receptors in asthma following the symposium 'New insights into the anti-inflammatory effects of A_{2A} adenosine receptor agonists' presented at the Life Sciences Meeting in Glasgow on 11th July, 2007. The potential role of A_{2A} agonists as a novel treatment for asthma is discussed in more detail by Dr Palmer and Dr Trevethick in this journal in a review entitled 'New insights into the anti-inflammatory effects of A_{2A} adenosine receptor agonists and antagonists: an introduction'. Furthermore, this article also provides an interesting comparison with adenosine receptors and cardiac inflammation as reviewed in this journal by Professor Joel Linden (University of Virginia, USA) in 'A_{2A} adenosine receptors in tissue reperfusion injury'.

Inhaled adenosine has long been recognized as a potent bronchoprovocant when administered to asthmatic but not healthy subjects, as first demonstrated by Cushley *et al.* (1983). However, although the effects of inhaled adenosine are well characterized, comparatively less is understood about the physiological role of endogenous extracellular adenosine in the airways and the pathological significance of an increase in the levels of this mediator in the airways. There is also a lack of information concerning the precise location and relative distribution of adenosine receptors in both healthy and asthmatic airways, although as virtually all cells express one or more of the adenosine receptor subtypes, there is a considerable amount of data pertaining to the function of adenosine receptors on a variety of cell types relevant to asthma. Consequently, a number of these subtype-specific cellular effects are currently being exploited to provide novel therapeutic opportunities for the treatment of asthma.

The aim of this review is to therefore discuss the current understanding of the role of endogenous adenosine in the pathophysiology of asthma, and thus the therapeutic basis of inhibiting certain receptor subtype-specific effects of adenosine, in particular the A_1 and A_{2B} receptors. Conversely, the rationale for activating subtype-specific adenosine receptor pathways, namely the A_{2A} pathway, as an alternative therapeutic approach will also be reviewed.

Correspondence: Professor CP Page, GKT School of Biomedical Sciences, Sackler Institute of Pulmonary Pharmacology, 5th floor Hodgkin Building, Guy's Campus, London Bridge, London SE1 1UL, UK.
E-mail: clive.page@kcl.ac.uk
Received 20 August 2007; revised 16 November 2007; accepted 13 December 2007

Adenosine receptors

The biological action of adenosine is mediated predominantly through specific cell surface receptors of which four subtypes (A_1, A_{2A}, A_{2B} and A_3) have been described (reviewed by Ralevic and Burnstock, 1998). Adenosine receptors are G-protein coupled 7-transmembrane receptors, with the A_1 and A_3 subtypes predominantly coupled to $G_{i/o}$ and the A_{2A} and A_{2B} to G_s, thus lowering and elevating the level of intracellular cAMP, respectively (Table 1). Binding studies revealed that the A_1 and A_{2A} subtypes have the highest affinity for adenosine, whereas the A_{2B} and A_3 receptors have a significantly lower affinity (Fredholm *et al.*, 2001). Adenosine receptors are ubiquitously expressed throughout the body, with virtually all cells expressing one or more adenosine receptor subtype. The location and identified functions of adenosine receptors on cells important in asthma are described in Table 2. With respect to the lung, little is known about the relative expression of adenosine receptor subtypes; however, binding studies in healthy peripheral lung tissue have suggested that A_2 receptor subtypes are much more abundant than the A_1 and A_3 receptor subtypes (Joad, 1990).

Table 1 G-protein coupling and signal transduction mechanisms of adenosine receptor subtypes

	A_1	A_{2A}	A_{2B}	A_3
G-protein	G_i/G_o	G_s/G_{olf}	$G_s/G_{q/11}$	$G_i/G_{q/11}$
Signal transduction	↓cAMP ↑IP_3/DAG	↑cAMP	↑cAMP ↑IP_3/DAG	↓cAMP ↑IP_3/DAG
Affinity for adenosine	High	High	Low	Low

Source and physiological role of endogenous adenosine

The nucleotide ATP has numerous biological functions, but it is best known as the primary source of free energy for metabolic processes within all living cells. ATP consists of a purine base (adenine) attached to the 1′ carbon atom of ribose and three phosphate groups attached at the 5′ carbon atom of the ribose (Figure 1). The liberation of the terminal phosphate group by hydrolysis is a highly exergonic reaction

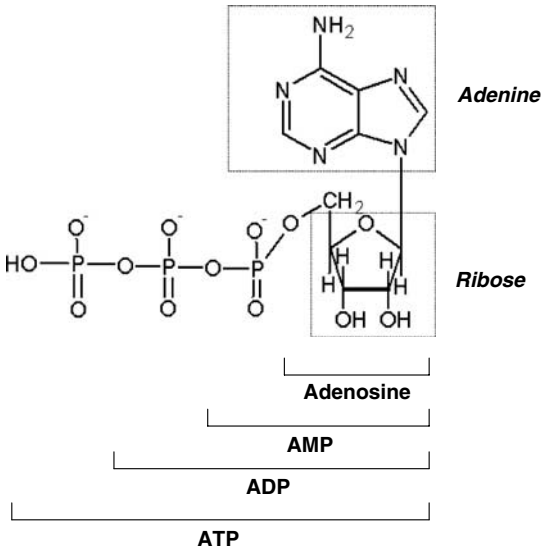

Figure 1 The structure of ATP and ATP derivatives.

Table 2 Expression and putative functions of adenosine receptor subtypes on cells involved in asthma

	A_1	A_{2A}	A_{2B}	A_3	*Putative functions*
Neutrophils	✓	✓		✓	A_1 induces degranulation, respiratory burst, chemotaxis, endothelial adherence A_{2A} is linked to inhibition of degranulation, respiratory burst, endothelial adhesion A_3 inhibits degranulation
Eosinophils	✓	✓		✓	A_1 is linked to eosinophil activation A_{2A} is linked to inhibition of respiratory burst A_3 is linked to inhibition of degranulation, respiratory burst, chemotaxis
Basophils		✓			A_{2A} inhibits leukotriene and histamine release
Mast cells		✓	✓		A_{2A} inhibits degranulation A_{2B} induces/promotes degranulation, cytokine secretion, IgE synthesis
T lymphocytes		✓	✓		A_{2A} inhibits activation and expansion A_{2B} inhibits activation
B lymphocytes		✓			Adenosine indirectly promotes IgE synthesis through mast cell activation
Monocytes	✓	✓	✓	✓	A_1 promotes phagocytosis A_{2A} inhibits IL-12 and TNF-α secretion, induces IL-10 and VEGF secretion
Platelets		✓			A_{2A} inhibits aggregation
Macrophages		✓	✓	✓	A_{2A} inhibits IL-12 and TNF-α secretion, NO production and induces IL-10 secretion A3 inhibits respiratory burst
Dendritic cells	✓	✓		✓	A_1 and A_3 promote chemotaxis (immature cells) A_{2A} inhibits IL-12 secretion (mature cells)
Bronchial epithelial cells	✓	✓	✓		A_1 induces mucin secretion A_{2A} stimulates wound healing A_{2B} induces IL-19 secretion
Bronchial smooth muscle	✓		✓		A_1 inhibits contraction A_{2B} induces IL-6 and MCP-1 secretion
Fibroblasts			✓		A_{2B} induces IL-6 secretion and differentiation into myofibroblasts
Endothelial cells	✓	✓	✓		A_{2A} inhibits IL-6 and IL-8 secretion and E-selectin and VCAM-1 expression from LPS-stimulated HUVECS; stimulates neo-vascularisation A_{2B} induces VEGF and IL-8 secretion

yielding ADP and inorganic phosphate. Under certain conditions such as high energy demand, ADP is also hydrolysed to AMP and subsequently the nucleoside adenosine through the action of intracellular 5′-nucleotidase (reviewed by Fredholm *et al*., 2001). As many cells express bidirectional nucleoside transporters (Cass *et al*., 1998), adenosine is able to cross the membrane and enter the extracellular space. Indeed, when the intracellular concentration of adenosine is very high, it can also passively diffuse out of the cell. ATP also functions as a neurotransmitter in both the central and peripheral nervous system, thus generating an extracellular source of adenosine following exposure to ectonucleotidases (reviewed by Burnstock, 2006).

Many of the diverse effects of extracellular adenosine upon physiological processes have now been characterized and include neurotransmission, vascular smooth muscle dilation and modulation of cardiac, renal, immune and inflammatory functions. Although the constitutively low extracellular concentration of adenosine is normally strictly regulated various metabolically stressful conditions such as hypoxia, ischaemia and inflammation can all cause the level to dramatically increase as a result of increased intracellular energy demand (Fredholm *et al*., 2001). An acutely elevated level of extracellular adenosine is considered to mediate anti-inflammatory and protective effects, and for this reason, adenosine has been labelled a 'retaliatory metabolite' (Newby, 1984). Probably the best known example of this is in the ischaemic hypoxic heart, whereby the increase in extracellular adenosine is cardioprotective by virtue of its vasodilatory and negative inotropic effects (Mubagwa *et al*., 1996). However, in a similar manner to many biological processes intended to be protective only in the short term, prolonged activity frequently generates detrimental consequences. Thus, with respect to asthma, chronic accumulation of adenosine in the airways is now becoming closely associated with various pathological aspects of the disease (Spicuzza *et al*., 2006).

Endogenous and exogenous adenosine in asthma

In asthmatic subjects, it has been demonstrated that adenosine levels in bronchoalveolar lavage fluid and exhaled breath condensate are significantly higher than those present in healthy subjects (Driver *et al*., 1993; Huszar *et al*., 2002). The study by Driver *et al*. (1993) estimated the concentration of adenosine in epithelial periciliary fluid to be approximately 60 μM in healthy subjects and 200 μM in asthmatic subjects. Furthermore, it has been observed that plasma adenosine levels rapidly increase following allergen challenge in asthmatic subjects (Mann *et al*., 1986) and are also increased in both plasma and exhaled breath condensate during exercise-induced bronchoconstriction (Vizi *et al*., 2002; Csoma *et al*., 2005).

The effects of exogenous inhaled AMP upon lung function in asthmatic subjects are very well characterized, and in addition some pro-inflammatory consequences of AMP challenge have been reported (AMP is used in preference to adenosine for solubility reasons and is rapidly hydrolysed to adenosine by 5′-ectonucleotidase in the lung). This knowledge therefore provides valuable insight into the significance of endogenous adenosine accumulation in asthma and the reported increase associated with an exacerbation. Thus, by considering what is currently understood about the effects of exogenously administered adenosine in asthmatic subjects, in addition to the experimental effects of adenosine upon cells *in vitro* and in animal models of allergic airway inflammation, we can begin to delineate the contribution of the endogenous accumulation of adenosine to the pathology of asthma.

Bronchoconstrictive effects of exogenous adenosine in asthmatic subjects

Firstly, the inhalation of AMP elicits a concentration-dependent bronchoconstriction. The first study to demonstrate this also showed that the chemically similar nucleoside guanosine had no effect (Cushley *et al*., 1983), nor did the deaminated adenosine metabolite inosine (Mann *et al*., 1986), suggesting that this was a specific adenosine receptor-mediated effect. The fall in forced expiratory volume in one second (FEV_1) is similar in duration (but somewhat less potent in molar terms) to that observed with methacholine and histamine, which all induce bronchoconstriction significantly faster than that which occurs following allergen challenge. As the response to AMP correlates more closely with the airway inflammation than methacholine or histamine challenge, AMP challenge is increasingly used as a non-invasive tool to assess disease severity and the response to anti-inflammatory therapy (Spicuzza and Polosa, 2003). Although inhalation of AMP does not generate a 'late response' as observed with allergen challenge, it has been suggested that adenosine induces mast cell degranulation via activation of the adenosine A_{2B} receptor subtype, rather than causing bronchoconstriction by acting directly on the airway smooth muscle (reviewed by Holgate, 2005). For this reason, adenosine has been classed as an 'indirect bronchoprovocant'. However, the mast cell/A_{2B} receptor theory has not been unequivocally proven due to the technical difficulties in obtaining a pure population of asthmatic airway mast cells, and the conclusions have thus been drawn from largely circumstantial evidence, which is therefore subject to alternative interpretations.

Nevertheless, the strongest evidence that has led to the conclusion of mast cell degranulation occurring in response to administration of adenosine was that obtained from pharmacological modulation of the asthmatic response to inhaled AMP. Pre-treatment with inhibitors of various bronchoconstrictive mast cell products including second-generation selective histamine H_1 receptor antagonists (Phillips *et al*., 1987; Rafferty *et al*., 1987a, b, Phillips and Holgate, 1989), cysteinyl leukotriene receptor antagonists (Rorke *et al*., 2002) and cyclooxygenase inhibitors (Crimi *et al*., 1989; Phillips and Holgate, 1989), all attenuated AMP-induced bronchoconstriction, as determined by an increased AMP PC_{20} (provocative concentration required to reduce baseline forced expiratory volume in one second by 20%) value in the presence of these drugs. In addition,

pre-treatment with the so-called 'mast cell stabilizers' nedocromil sodium and disodium cromoglycate also inhibited AMP-induced bronchoconstriction (Crimi *et al*., 1988; Phillips *et al*., 1989; Summers *et al*., 1990). In support of these studies, Polosa *et al*. (1995) demonstrated the release of mast cell-derived mediators following endobronchial adenosine challenge in asthmatic subjects.

However, these observations can be interpreted in several other ways. Firstly, adenosine receptors are ubiquitously expressed and, given the inflammatory milieu in asthmatic airways, it is possible that adenosine is stimulating the release of inflammatory mediators from other cells, which then promote degranulation of tissue mast cells. Furthermore, the absence of a 'late response' suggests that the mast cells are not degranulating in the same manner as that which occurs following allergen challenge, and also mast cells are not an exclusive cellular source of histamine, leukotrienes and arachidonic acid derivatives. Secondly, Bjorck *et al*. (1992) investigated the effects of adenosine on isolated human bronchial strips and demonstrated a variable but overall small contractile response in tissue from healthy subjects, which was significantly increased in tissue from asthmatic subjects closely reflecting the clinical situation. The adenosine-induced contraction observed with tissue from asthmatic subjects was significantly inhibited by the A_1 receptor antagonist 2-thio-((1,3-dipropyl)-8-cyclopentyl)-xanthine, suggesting it was A_1 receptor mediated, an adenosine receptor subtype that mast cells are not considered to express, and therefore the contractile response in tissue from subjects with asthma did not involve the A_{2B} receptor. However, the same study also showed that the response could be attenuated significantly by pre-incubation with an H_1 receptor antagonist and leukotriene synthesis inhibitor. A subsequent investigation by Ellis and Undem (1994) demonstrated that isolated human bronchial tissue has a high degree of intrinsic tone and that histamine and leukotrienes constitute a major part of this basal tone, inhibition of which potently relaxes the tissue, suggesting further that the results of Bjorck were probably not mediated via mast cell degranulation. Furthermore, it has been suggested that histamine and leukotrienes contribute to bronchial tone in subjects with asthma, as it is recognized that a single-dose administration of both H_1 receptor antagonists and leukotriene receptor antagonists can increase baseline FEV_1 (Reiss *et al*., 1997; Dekhuijzen and Koopmans, 2002; Nelson, 2003). Thus, it is difficult to ascertain precisely up to what degree the inhibition of adenosine-induced contractile responses by the H_1 receptor antagonist and leukotriene synthesis inhibitor in both the *in vitro* and clinical setting can be attributed to the direct inhibition of mast cell products released by adenosine or to an altered intrinsic tone. The possibility that inhibiting mast cell products in asthmatic subjects increases the PC_{20} to inhaled AMP partly through physiological antagonism should therefore be considered. Although the studies described above investigating pharmacological modulation of the AMP response did not observe significant changes in PC_{20} values to the other bronchoprovocants concurrently tested (with the exception of the corresponding agonist), it is possible that the bronchoconstrictive effects of AMP could be significantly more sensitive to the presence of pharmacological agents acting as a physiological antagonist as demonstrated with β_2 agonists (Phillips *et al*., 1990; Nightingale *et al*., 1999). In support of this, the first-generation H_1 receptor antagonist chlorpromazine had no effect upon AMP challenge (Crimi *et al*., 1986), and the direct bronchodilatory effect of a single dose of first-generation H_1 receptor antagonists was subsequently demonstrated to be less efficacious than that of second-generation H_1 receptor antagonists (Wood-Baker and Holgate, 1993). Furthermore, second-generation H_1 receptor antagonists have been reported to also possess anti-inflammatory effects unrelated to histamine inhibition (Lever *et al*., 2007; reviewed by Walsh, 2002), which may also affect the response to AMP. Likewise, a number of non-mast cell effects of nedocromil sodium and disodium cromoglycate have also been described, particularly effects on airway sensory nerves (Barnes, 2006).

A very recent study (Liesker *et al*., 2007) should also be taken into consideration, as this does not support the involvement of mast cell degranulation in response to AMP challenge. This study attempted to compare mast cell density within smooth muscle regions in bronchial biopsy material obtained from healthy, asthmatic and chronic obstructive pulmonary disease (COPD) subjects and to investigate whether the number of mast cells correlated with AMP responsiveness. No differences in mast cell numbers were observed between the three subject groups, leading the authors to conclude that mast cells are not important in the bronchoconstrictor response to AMP.

Nevertheless, it was suggested that bronchoconstriction to adenosine was mediated through the A_{2B} receptor subtype (reviewed by Holgate, 2005), as the methylxanthine enprofylline, which has a weak but selective antagonistic effect at A_{2B} receptors, attenuated the effects of AMP challenge (Clarke *et al*., 1989). However, at the same dose, enprofylline also inhibited the response to histamine challenge to a very similar extent, suggesting a shared inhibitory mechanism, most likely physiological antagonism resulting from phosphodiesterase enzyme inhibition. Interestingly, in the same study, it was demonstrated that theophylline, which is an antagonist at all adenosine receptor subtypes, inhibited the response to histamine in a similar manner to enprofylline, yet was significantly more potent at inhibiting the effects of AMP than histamine, suggesting the presence of an additional inhibitory mechanism. It can be concluded from this study, therefore, that antagonism of adenosine receptors other than the A_{2B} receptor was more efficacious at inhibiting the AMP-induced bronchoconstriction. Furthermore, a very recent study demonstrated a total lack of effect of a selective dual A_{2B}/A_3 receptor antagonist upon AMP PC_{20} in asthmatic subjects (Pascoe *et al*., 2007), suggesting that A_{2B} or indeed the A_3 receptors are not involved in this response.

The *in vitro* evidence implicating mast cell degranulation in response to adenosine in addition to suggesting that the A_{2B} receptor mediates this effect is also ambiguous. The most convincing evidence was reported by Forsythe *et al*. (1999), who demonstrated the release of histamine from human bronchoalveolar lavage mast cells following treatment with adenosine. The caveat to this study is that the mast cells were

not isolated from the other bronchoalveolar lavage cells, and so it is possible that adenosine was inducing inflammatory mediator release from different cells, which then promoted mast cell degranulation. An earlier study with primary human mast cells demonstrated that adenosine can potentiate mast cell degranulation induced by other stimuli such as IgE and calcium ionophore, but was not able to act alone (Peachell *et al*., 1988) and indeed was also shown to inhibit degranulation (Hughes *et al*., 1984).

Other experimental approaches have focused upon the human mastocytoma-1 (HMC-1) cell line, which was derived from a patient with mast cell leukaemia (Butterfield *et al*., 1988). In summary, these studies have demonstrated that HMC-1 cells express the A_{2A}, A_{2B} and A_3 receptors and that adenosine can stimulate interleukin (IL)-8 secretion (Feoktistov and Biaggioni, 1995), in addition to IL-1β, IL-3, IL-4 and IL-13 secretion (Ryzhov *et al*., 2004), all via the A_{2B} receptor. Potentiation of inflammatory mediator secretion was also demonstrated with rat peritoneal mast cells (Marquardt *et al*., 1978) and murine bone marrow-derived mast cells (Marquardt *et al*., 1984). Thus, although these studies demonstrate that adenosine can certainly exert pro-inflammatory effects upon mast cells in the presence of other stimuli, most likely through activation of the A_{2B} receptor, none were able to demonstrate adenosine-induced degranulation per se.

In summary, the evidence to date suggests that it is not possible to definitely conclude that inhaled adenosine induces bronchoconstriction through mast cell degranulation, and alternative mechanisms should not be disregarded. The ability of adenosine to mediate bronchoconstriction through direct effects on airway smooth muscle as well as through neuronal mechanisms are two such other possibilities that ought to be considered.

In support of adenosine possessing direct bronchoconstrictor effects, it has been demonstrated that expression of the adenosine A_1 receptor is significantly elevated on airway smooth muscle obtained from asthmatic subjects in comparison to healthy subjects (Brown *et al*., 2008). Furthermore, culture of human airway smooth muscle cells in asthmatic serum was shown to upregulate A_1 receptor expression (Hakonarson *et al*., 1998). An increase in airway A_1 receptor expression was also observed in a rabbit model of allergic airway inflammation when compared to healthy naïve animals (Nyce and Metzger, 1997) and in adenosine-deaminase knockout mice, which have elevated extracellular adenosine levels (Chunn *et al*., 2001; Zhong *et al*., 2001). Moreover, the A_1 receptor was upregulated on airway smooth muscle in an allergic rabbit model, and when this expression was inhibited with the use of an antisense oligonucleotide directed against the A_1 receptor, the bronchoconstrictive response to inhaled adenosine was inhibited (Nyce and Metzger, 1997; Abebe and Mustafa, 1998). In addition, this was also demonstrated recently with a selective adenosine A_1 receptor antagonist (Obiefuna *et al*., 2005). Thus, as the A_1 receptor is G_i-protein coupled and therefore lowers cAMP following activation, it is likely that adenosine can directly contract airway smooth muscle, and in support of this, Ethier and Madison (2006) demonstrated that activation of the A_1 receptor on human airway smooth muscle cells *in vitro* induced calcium mobilization. The study described earlier by Bjorck *et al*. (1992) also demonstrated involvement of the A_1 receptor in mediating contraction of asthmatic bronchial tissue *in vitro*. By considering the possibility that adenosine acts directly on the muscle, the pharmacological modulation of AMP PC_{20} with histamine and leukotriene receptor antagonists described earlier could be attributed to an inhibition of the ability of histamine and leukotrienes to prime airway smooth muscle and thereby increasing the reactivity of muscle cells to various spasmogens (Lee *et al*., 1984; Jacques *et al*., 1991, 1992; Carbajal *et al*., 2005). Although various animal studies provide support for a role of adenosine A_1 receptors in mediating airway obstruction, it is acknowledged that species and methological differences account for the demonstration of a role for atypical (Hannon *et al*., 1998) A_{2B} and A_3 receptors in this response (Fan *et al*., 2003; Hua *et al*., 2007). Nevertheless, human data support a role for adenosine A_1 receptors in mediating airway smooth muscle contraction.

Evidence implicating a role for nerves in the asthmatic response to adenosine has also been described. One of the first clinical studies to suggest this resulted from observing the inhibitory effects of ipratropium bromide on AMP challenge, indicating a possible involvement of cholinergic neural pathways (Polosa *et al*., 1991). Furthermore, investigations into the effects of inhaled loop diuretics frusemide and bumetanide on AMP challenge revealed that they could attenuate the effects of AMP, which is thought to result from modifying sensory nerve activity (O'Connor *et al*., 1991; Polosa *et al*., 1993). Polosa *et al*. (1992) demonstrated that previous repeated challenges with inhaled bradykinin, which depletes neuropeptides from sensory nerve endings, significantly attenuated the response to AMP challenge. However, a subsequent study did not support this observation, as previous treatment of asthmatic subjects with phosphoramidon, a neutral endopeptidase inhibitor, failed to enhance bronchoconstrictor sensitivity to AMP (Polosa *et al*., 1997), which suggested that certain neuropeptides including neurokinin A and substance P may not play a significant role in the response to AMP. Some *in vivo* evidence does, however, suggest an involvement of sensory nerves; for example, the inhibition of AMP- and the selective A_1 receptor agonist cyclopentyl adenosine-induced airway obstruction by atropine, capsaicin and bilateral vagotomy in allergic guinea pigs suggests a neuronal-dependent mechanism with the particular involvement of capsaicin-sensitive nerves (Keir *et al*., 2006). In addition, a very recent study investigating the airway effects of adenosine in four strains of naïve mice, each lacking one of the four adenosine receptors, demonstrated that adenosine induces bronchoconstriction through the A_1 receptor, and that this was likely to be mediated through sensory nerves (Hua *et al*., 2007).

Pro-inflammatory effects of exogenous adenosine in asthmatic subjects

In addition to exogenous adenosine affecting airway tone, inhalation of AMP in asthmatic subjects has been shown to increase airway eosinophilia within 1 h of AMP challenge

(van den Berge *et al*., 2004), supporting earlier observations in an allergic guinea pig model (Spruntulis and Broadley, 2001), which was also accompanied by an increase in macrophage number and inhibited by an A_3 receptor antagonist. Incidentally, studies investigating adenosine receptors expressed on human eosinophils identified A_1, A_{2A} and A_3 subtypes, with the A_1 receptor linked to activation of the eosinophil and the A_{2A} and unexpectedly the A_3 receptor inhibiting eosinophil activation (Ezeamuzie and Philips, 1999, 2003) and chemotaxis (Knight *et al*., 1997). Indeed, the A_3 was found to be atypically positively coupled to adenylate cyclase, which explained these effects (Ezeamuzie and Philips, 2003). Furthermore, expression of A_3 receptors was upregulated in asthmatic lung tissue, where it was located predominantly to eosinophils (Walker *et al*., 1997). Thus, although the mechanism by which adenosine can increase airway eosinophilia in asthmatic subjects remains to be determined, these data suggest that adenosine may promote inflammatory leukocyte recruitment to the lung in subjects with asthma.

Given the evidence described above, the effects of exogenously administered adenosine in asthmatic subjects are all associated with recognized features of asthma. It is clear that although the precise mechanisms through which exogenous adenosine is able to induce bronchoconstriction remain to be fully elucidated, it can be speculated that the increase in extracellular adenosine during an asthmatic exacerbation contributes to the bronchoconstriction response to some extent; however, how significant a contribution this may be remains to be established. In addition, the evidence suggests that endogenous adenosine may promote inflammatory cell infiltration. Thus, taken together, these data advocate a role for extracellular endogenous adenosine in the pathophysiology in asthma. In support of this hypothesis, a number of experimental *in vitro* and *in vivo* studies provide further evidence advocating a role for adenosine in asthma, and will subsequently be described.

Putative effects of elevated endogenous adenosine in asthmatic subjects

Firstly, mucus hypersecretion is an important feature of asthma and adenosine has been shown to upregulate expression of the mucin gene, *MUC2*, in human tracheal epithelial cells *in vitro* through activation of the adenosine A_1 receptor (McNamara *et al*., 2004). In support of adenosine possessing secretagogue properties, it has been demonstrated that adenosine was able to induce canine tracheal mucus secretion *in vivo* through an A_1 receptor-dependent mechanism (Johnson and McNee, 1985).

Secondly, neutrophils are considered to play an important role in the pathophysiology of moderate-to-severe asthma (Jatakanon *et al*., 1999), and through activating the A_1 receptor, adenosine has been shown to promote human neutrophil adherence to endothelial cells (Cronstein *et al*., 1992) and chemotaxis (Cronstein *et al*., 1990), upregulate CD11b/CD18 expression (Wollner *et al*., 1993) and trigger the respiratory burst (Cronstein *et al*., 1985, 1987) and degranulation (Bouma *et al*., 1997). In addition, adenosine also promotes monocyte phagocytosis through the A_1 receptor (Salmon *et al*., 1993) and increases endothelial cell permeability (Wilson and Batra, 2002). Adenosine has been shown to induce IL-6 and MCP-1 secretion from human airway smooth muscle cells (Zhong *et al*., 2004), IL-19 from human bronchial epithelial cells (Zhong *et al*., 2006) and IL-6 from fibroblasts (Zhong *et al*., 2005), all through activation of the A_{2B} receptor, in addition to inducing the differentiation of fibroblasts into myofibroblasts. It should be noted, however, that many inflammatory cells express adenosine A_{2A} receptors, and activation of these will lead to suppression of cell functions (reviewed by Bours *et al*., 2006), as highlighted in Table 2. As inflammatory cells usually express more than one adenosine receptor subtype with varying affinity for adenosine, adenosine is capable of inducing signalling pathways that result in opposing cellular effects directly dependent upon the concentration. The overall effect, therefore, of adenosine in asthmatic airways upon local inflammatory cells remains to be determined.

Further to these studies, an important study unequivocally demonstrating the pathological respiratory effects of an increased extracellular adenosine level has been described in mice with a genetically engineered partial adenosine-deaminase deficiency, which reduces their ability to metabolize adenosine to inosine (Chunn *et al*., 2001). Consequently, these mice accumulate high concentrations of adenosine extracellularly and exhibit a marked pulmonary inflammation. Some of the pathological features reported in this model were consistent with those observed in asthma such as mucus hypersecretion, but the pulmonary inflammatory cell profile differed considerably and consisted mainly of macrophages, with no increase in eosinophil numbers. However, transcript levels for the A_1, A_{2B} and A_3 receptors were found to be significantly increased, suggesting that an increase in extracellular adenosine promotes adenosine receptor signalling. Mice totally deficient in adenosine deaminase displayed more severe pulmonary inflammation and died at 3 weeks of age from respiratory distress (Blackburn *et al*., 2000). Additional inflammatory features observed included pulmonary and peripheral blood eosinophilia, extensive mast cell degranulation and an increase in serum IgE (Zhong *et al*., 2001). Exogenous administration of adenosine deaminase could both prevent and reverse the lung inflammation and damage observed in these genetically altered animals, suggesting that the increased extracellular adenosine was mediating these effects. Interestingly, concurrent knockout of the adenosine A_1 receptor was reported to exacerbate the inflammation further, suggesting a protective role of the A_1 receptor in this model (Sun *et al*., 2005). However, the levels of eosinophils in this model were of a magnitude unlikely to result in alterations in airway responsiveness, and therefore the relevance of these findings to asthma should be interpreted with caution (less than 2% of total cells, compared with approximately 40% of total in a murine model of allergic inflammation (Riffo-Vasquez *et al*., 2004)).

Interestingly, a subset of patients with severe combined immunodeficiency disease is deficient in adenosine deaminase, and consequently plasma adenosine levels are elevated. An increased incidence of various respiratory disorders

including asthma have been observed in a significant number of these patients, in addition to features associated with asthma such as peripheral blood eosinophilia and elevated serum IgE (reviewed by Blackburn and Kellems, 2005). Furthermore, subjects with COPD also have an increased extracellular level of adenosine in the airways, which provides further evidence of the association between increased adenosine and pulmonary disease (Sullivan *et al.*, 2005). Inhaled AMP can also induce bronchoconstriction in COPD patients, although not to the same extent as in asthmatic subjects. Interestingly however, it has very recently been demonstrated that subjects with COPD have an increased density of A_1, A_{2A} and A_3 receptors in the lung parenchyma when compared to age-matched smoking controls (Varani *et al.*, 2006), although the associated functions remain to be determined.

In conclusion, the clinical and experimental evidence described above suggests that the chronic accumulation of extracellular adenosine in asthmatic airways may contribute towards the pathogenesis and/or pathophysiology of asthma. Consequently, adenosine receptors have become attractive therapeutic targets for the treatment of asthma. Furthermore, as many cells express more than one subtype of adenosine receptor, activation of which can have opposing cellular effects, developing selective agonists that induce a specific effect on a particular cell type are also under investigation.

Adenosine receptors as targets for the treatment of asthma

The A_1, A_{2A} and A_{2B} receptors are the target of various drugs in development for the treatment of asthma. Pre-clinical evidence supports the rationale for the use of antagonists at the A_1 and A_{2B} receptors and an agonist at the A_{2A} receptor. The role of the A_3 receptor in asthma has yet to be clarified. Thus, it remains to be determined whether it is of greater therapeutic benefit to inhibit the effects of extracellular adenosine in asthma with selective antagonists against the A_1 and A_{2B} receptors, or to exploit the inhibitory cellular events downstream of the A_{2A} receptor with an A_{2A} selective agonist.

Adenosine A_1 receptor

The rationale for targeting the adenosine A_1 receptor is based on the evidence described above obtained from *in vitro* and *in vivo* studies in addition to clinical findings. In summary, by activating the A_1 receptor, adenosine has been shown to mediate bronchoconstriction, mucin production, promote pro-inflammatory functions of neutrophils and monocytes and increase endothelial cell permeability. Thus, to inhibit adenosine A_1 receptor-mediated pathways, two distinct approaches have been taken.

The first approach was to develop an inhaled antisense oligonucleotide against the A_1 receptor, as the therapeutic efficacy of this approach was demonstrated in the pivotal study by Nyce and Metzger (1997), in a rabbit model of allergic airway inflammation. Antisense oligonucleotides block mRNA and therefore protein expression of the targeted gene. The oligonucleotide used in this study, EPI-2010 (EpiGenesis Pharmaceuticals, New Jersey, USA), was also shown to significantly increase AMP PC_{20} in a primate model of asthma (Nyce, 1999). The main advantages of inhaled antisense oligonucleotides over small-molecule antagonists are a lower incidence of systemic side effects, which is particularly important given the physiological functions of A_1 receptors in other organs, in addition to a longer half-life (once-weekly dosing) and greater specificity for the desired target. Thus, given the pre-clinical data, the antisense oligonucleotide against A_1 receptor mRNA EPI-2010 was investigated as an inhaled therapy for the treatment of asthma in humans. Although well tolerated in Phase I, the Phase IIa trial demonstrated that treatment with EPI-2010 yielded only modest improvements in asthmatic subjects, which were not deemed sufficient to justify continuing the clinical development of this therapy (reviewed by Ball *et al.*, 2004).

The second approach reverted to more traditional methods and has involved the development of a small-molecule A_1 receptor antagonist, L-97-1, for oral administration (Obiefuna *et al.*, 2005). This has proved successful in pre-clinical models at attenuating both adenosine and allergen-induced bronchoconstriction, in addition to airway inflammation (Nadeem *et al.*, 2006). As it is based very closely upon the methylxanthine bamiphylline, which has been approved for the treatment of respiratory diseases for a number of years, serious adverse effects are not anticipated.

Adenosine A_{2A} receptor

In comparison to the A_1 receptor, there has been significantly more interest in developing selective A_{2A} receptor agonists for the treatment of respiratory diseases and other chronic inflammatory conditions. Most inflammatory cells express the A_{2A} receptor, and being G_s-protein coupled, activation almost always elicits inhibitory effects, as described in Table 2 (reviewed by Lappas *et al.*, 2005). To summarize the observations most relevant to asthma, selective activation of the A_{2A} receptor inhibits histamine and tryptase release from mast cells (Hughes *et al.*, 1984; Peachell *et al.*, 1991; Suzuki *et al.*, 1998), neutrophil adherence to the endothelium (Cronstein *et al.*, 1992; Felsch *et al.*, 1995), integrin upregulation (Wollner *et al.*, 1993) and the respiratory burst (Fredholm *et al.*, 1996; Hannon *et al.*, 1998) and degranulation (Bouma *et al.*, 1997). In addition, activation of the A_{2A} receptor inhibits IL-12 and TNF-α release from activated monocytes (Bouma *et al.*, 1994; Zhang *et al.*, 2005) and enhances IL-10 and vascular endothelial growth factor secretion (reviewed by Hasko *et al.*, 2007), in addition to inhibiting IL-12 secretion from mature dendritic cells (Panther *et al.*, 2001). Furthermore, the A_{2A} receptor has been demonstrated to suppress the activation and expansion of T lymphocytes (Huang *et al.*, 1997). In bronchial epithelial cells, the A_{2A} receptor stimulated wound healing (Allen-Gipson *et al.*, 2006). In Brown-Norway rats sensitized to ovalbumin, administration of an A_{2A} agonist CGS-21680

attenuated the airway inflammation induced by ovalbumin challenge with a magnitude similar to that achieved by budesonide (Fozard *et al*., 2002). Finally, in a murine model of allergic airway inflammation, genetic deletion of the A_{2A} receptor augmented the airway inflammation and hyperresponsiveness (Nadeem *et al*., 2007), further supporting the role of the A_{2A} receptor as mediating anti-inflammatory functions.

Thus, by exerting inhibitory effects on multiple inflammatory cell types, it has been predicted that a selective A_{2A} receptor agonist may have broad-spectrum anti-inflammatory effects similar to glucocorticosteroids. Several pharmaceutical companies now have A_{2A} agonists in clinical development for other inflammatory conditions such as COPD, but it is expected that they will be investigated for their efficacy in treating asthma, for example, UK-432097 (Pfizer, Sandwich, UK). A GlaxoSmithKline A_{2A} agonist reached Phase II for the treatment of asthma (GW328267), but was discontinued, possibly due to a lack of efficacy (Luijk *et al*., 2003). Whether this was due to the pharmacokinetic profile of the compound, or simply that A_{2A} agonists are not as beneficial as predicated, remains to be established.

Adenosine A_{2B} receptor

The therapeutic potential of an antagonist at the A_{2B} receptor is based largely on the cellular effects downstream of this receptor in mast cells, as described earlier, which enhance the release of inflammatory mediators in addition to pro-inflammatory effects on airway smooth muscle cells, epithelial cells and fibroblasts. Data obtained from various animal models of allergic airway inflammation suggest that inhibition of A_{2B}-mediated effects may be of therapeutic benefit in asthma (Fozard *et al*., 2003). A very recent study using a murine model by Mustafa *et al*. (2007) demonstrated that the selective A_{2B} receptor antagonist CVT-6883 (CV Therapeutics, Palo Alto, USA) attenuated the bronchoconstriction induced by inhaled AMP and allergen challenge in addition to the inflammatory cell infiltration.

Thus, CVT-6883 along with various other compounds, for example, CGS15493 (Novartis, Basel, Switzerland), WO-00125210 (Bayer Shering Pharma), IPDX (Vanderbilt University, Nashville, USA), ATL-907 (Adenosine Therapeutics, Charlottesville, USA), are being investigated for the treatment of asthma, some of which are now in Phase I and Phase II trials. A dual A_{2B}/A_3 antagonist QAF805 (Novartis, Basel, Switzerland) recently reached Phase II clinical trial, although it demonstrated no effect on AMP PC_{20} in mild asthmatic subjects (Pascoe *et al*., 2007).

Adenosine A_3 receptor

The role of the A_3 receptor in the human lung and indeed in asthma remains to be clarified. What is clear, however, is that the expression of the A_3 receptor in asthmatic airways is predominantly located in eosinophils, and *in vitro* studies have demonstrated that the A_3 receptor mediates inhibition of eosinophil chemotaxis and activation. Thus, the elevated level of adenosine in asthmatic airways may be protective through activating the A_3 receptor. Enhancing the effects, therefore, of endogenous adenosine with a selective A_3 receptor agonist may be beneficial in the treatment of asthma. Conversely, it has been suggested that an A_3 receptor antagonist may be useful in the treatment of asthma, as data obtained from animal models demonstrate that the A_3 receptor can mediate mast cell degranulation, bronchoconstriction, mucus secretion and eosinophilia (Ramkumar *et al*., 1993; Fan *et al*., 2003; Tilley *et al*., 2003; Young *et al*., 2004). However, these conflicting data only serve to highlight the necessity of further investigations concerning the role of the A_3 receptor in asthma, before considering the therapeutic potential of targeting this receptor in the treatment of asthma although with an agonist or antagonist.

Conclusion

Asthma remains a disease with unmet therapeutic needs. The evidence to date strongly suggests that adenosine contributes to the pathophysiology of asthma, although the precise extent remains to be determined. Pre-clinical evidence, however, certainly warrants the current investigations into the adenosine A_1 and A_{2B} receptor subtypes as novel therapeutic targets for the treatment of asthma. In addition, stimulating A_{2A} receptor-mediated cellular pathways remains an exciting prospect, given the potential broad-spectrum activity such a drug may yield. Furthermore, the combination of both therapeutic approaches may provide an even better way of modifying adenosine receptors as a treatment for asthma.

Conflict of interest

The authors state no conflict of interest.

References

Abebe W, Mustafa SJ (1998). A_1 adenosine receptor-mediated $Ins(1,4,5)P_3$ generation in allergic rabbit airway smooth muscle. *Am J Physiol* **275**: L990–L997.

Allen-Gipson DS, Wong J, Spurzem JR, Sisson JH, Wyatt TA (2006). Adenosine A_{2A} receptors promote adenosine-stimulated wound healing in bronchial epithelial cells. *Am J Physiol Lung Cell Mol Physiol* **290**: L849–L855.

Ball HA, Van Scott MR, Robinson CB (2004). Sense and antisense: therapeutic potential of oligonucleotides and interference RNA in asthma and allergic disorders. *Clin Rev Allergy Immunol* **27**: 207–217.

Barnes PJ (2006). Drugs for asthma. *Br J Pharmacol* **147**: S297–S303.

Bjorck T, Gustafsson LE, Dahlen SE (1992). Isolated bronchi from asthmatics are hyperresponsive to adenosine, which apparently acts indirectly by liberation of leukotrienes and histamine. *Am Rev Respir Dis* **145**: 1087–1091.

Blackburn MR, Kellems RE (2005). Adenosine deaminase deficiency: metabolic basis of immune deficiency and pulmonary inflammation. *Adv Immunol* **86**: 1–41.

Blackburn MR, Volmer JB, Thrasher JL, Zhong H, Crosby JR, Lee JJ *et al*. (2000). Metabolic consequences of adenosine deaminase deficiency in mice are associated with defects in alveogenesis,

pulmonary inflammation, and airway obstruction. *J Exp Med* **192**: 159–170.

Bouma MG, Jeunhomme TM, Boyle DL, Dentener MA, Voitenok NN, van den Wildenberg FA *et al*. (1997). Adenosine inhibits neutrophil degranulation in activated human whole blood: involvement of adenosine A_2 and A_3 receptors. *J Immunol* **158**: 5400–5408.

Bouma MG, Stad RK, van den Wildenberg FA, Buurman WA (1994). Differential regulatory effects of adenosine on cytokine release by activated human monocytes. *J Immunol* **153**: 4159–4168.

Bours MJ, Swennen EL, Di Virgilio F, Cronstein BN, Dagnelie PC (2006). Adenosine 5′-triphosphate and adenosine as endogenous signaling molecules in immunity and inflammation. *Pharmacol Ther* **112**: 358–404.

Brown RA, Clarke GW, Ledbetter CL, Hurle MJ, Denyer JC, Simcock DE *et al*. (2008). Elevated expression of adenosine A_1 receptor in bronchial biopsy specimens from asthmatic subjects. *Eur Respir J* **31**: 1–9.

Burnstock G (2006). Historical review: ATP as a neurotransmitter. *Trends Pharmacol Sci* **27**: 166–176.

Butterfield JH, Weiler D, Dewald G, Gleich GJ (1988). Establishment of an immature mast cell line from a patient with mast cell leukemia. *Leuk Res* **12**: 345–355.

Carbajal V, Vargas MH, Flores-Soto E, Martínez-Cordero E, Bazán-Perkins B, Montaño LM (2005). LTD4 induces hyperresponsiveness to histamine in bovine airway smooth muscle: role of SR-ATPase Ca2 + pump and tyrosine kinase. *Am J Physiol Lung Cell Mol Physiol* **288**: L84–L92.

Cass CE, Young JD, Baldwin SA (1998). Recent advances in the molecular biology of nucleoside transporters of mammalian cells. *Biochem Cell Biol* **76**: 761–770.

Chunn JL, Young HWJ, Banerjee SK, Colasurdo GN, Blackburn MR (2001). Adenosine-dependent airway inflammation and hyperresponsiveness in partially adenosine deaminase-deficient mice. *J Immunol* **167**: 4676–4685.

Clarke H, Cushley MJ, Persson CG, Holgate ST (1989). The protective effects of intravenous theophylline and enprofylline against histamine- and adenosine 5′-monophosphate-provoked bronchoconstriction: implications for the mechanisms of action of xanthine derivatives in asthma. *Pulm Pharmacol* **2**: 147–154.

Crimi E, Brusasco V, Brancatisano M, Losurdo E, Crimi P (1986). Adenosine-induced bronchoconstriction: premedication with chlorpheniramine and nedocromil sodium. *Eur J Respir Dis Suppl* **147**: 255–257.

Crimi N, Palermo F, Oliveri R, Vancheri C, Polosa R, Palermo B *et al*. (1988). Comparative study of the effects of nedocromil sodium (4 mg) and sodium cromoglycate (10 mg) on adenosine-induced bronchoconstriction in asthmatic subjects. *Clin Allergy* **18**: 367–374.

Crimi N, Palermo F, Polosa R, Oliveri R, Maccarrone C, Palermo B *et al*. (1989). Effect of indomethacin on adenosine-induced bronchoconstriction. *J Allergy Clin Immunol* **83**: 921–925.

Cronstein BN, Daguma L, Nichols D, Hutchison AJ, Williams M (1990). The adenosine/neutrophil paradox resolved: human neutrophils possess both A1 and A2 receptors that promote chemotaxis and inhibit O2 generation, respectively. *J Clin Invest* **85**: 1150–1157.

Cronstein BN, Kubersky SM, Weissmann G, Hirschhorn R (1987). Engagement of adenosine receptors inhibits hydrogen peroxide (H_2O_2-) release by activated human neutrophils. *Clin Immunol Immunopathol* **42**: 76–85.

Cronstein BN, Levin RI, Philips M, Hirschhorn R, Abramson SB, Weissmann G (1992). Neutrophil adherence to endothelium is enhanced via adenosine A_1 receptors and inhibited via adenosine A_2 receptors. *J Immunol* **148**: 2201–2206.

Cronstein BN, Rosenstein ED, Kramer SB, Weissmann G, Hirschhorn R (1985). Adenosine; a physiologic modulator of superoxide anion generation by human neutrophils. Adenosine acts via an A2 receptor on human neutrophils. *J Immunol* **135**: 1366–1371.

Csoma Z, Huszar E, Vizi E, Vass G, Szabo Z, Herjavecz I *et al*. (2005). Adenosine level in exhaled breath increases during exercise-induced bronchoconstriction. *Eur Respir J* **25**: 873–878.

Cushley MJ, Tattersfield AE, Holgate ST (1983). Inhaled adenosine and guanosine on airway resistance in normal and asthmatic subjects. *Br J Clin Pharmacol* **15**: 161–165.

Dekhuijzen PN, Koopmans PP (2002). Pharmacokinetic profile of zafirlukast. *Clin Pharmacokinet* **41**: 105–114.

Driver AG, Kukoly CA, Ali S, Mustafa SJ (1993). Adenosine in bronchoalveolar lavage fluid in asthma. *Am Rev Respir Dis* **148**: 91–97.

Ellis JL, Undem BJ (1994). Role of cysteinyl-leukotrienes and histamine in mediating intrinsic tone in isolated human bronchi. *Am J Respir Crit Care Med* **149**: 118–122.

Ethier MF, Madison JM (2006). Adenosine A_1 receptors mediate mobilization of calcium in human bronchial smooth muscle cells. *Am J Respir Cell Mol Biol* **35**: 496–502.

Ezeamuzie CI, Philips E (1999). Adenosine A_3 receptors on human eosinophils mediate inhibition of degranulation and superoxide anion release. *Br J Pharmacol* **127**: 188–194.

Ezeamuzie CI, Philips E (2003). Positive coupling of atypical adenosine A_3 receptors on human eosinophils to adenylyl cyclase. *Biochem Biophys Res Commun* **300**: 712–718.

Fan M, Qin W, Mustafa SJ (2003). Characterization of adenosine receptor(s) involved in adenosine-induced bronchoconstriction in an allergic mouse model. *Am J Physiol Lung Cell Mol Physiol* **284**: L1012–L1019.

Felsch A, Stocker K, Borchard U (1995). Phorbol ester-stimulated adherence of neutrophils to endothelial cells is reduced by adenosine A_2 receptor agonists. *J Immunol* **155**: 333–338.

Feoktistov I, Biaggioni I (1995). Adenosine A_{2b} receptors evoke interleukin-8 secretion in human mast cells. An enprofylline-sensitive mechanism with implications for asthma. *J Clin Invest* **96**: 1979–1986.

Forsythe P, Ennis M (1999). Adenosine, mast cells and asthma. *Inflamm Res* **48**: 301–307.

Forsythe P, McGarvey LP, Heaney LG, MacMahon J, Ennis M (1999). Adenosine induces histamine release from human bronchoalveolar lavage mast cells. *Clin Sci (Lond)* **96**: 349–355.

Fozard JR, Baur F, Wolber C (2003). Antagonist pharmacology of adenosine A_{2B} receptors from rat, guinea pig and dog. *Eur J Pharmacol* **475**: 79–84.

Fozard JR, Ellis KM, Villela Dantas MF, Tigani B, Mazzoni L (2002). Effects of CGS21680, a selective adenosine A_{2A} receptor agonist, on allergic airways inflammation in the rat. *Eur J Pharmacol* **438**: 183–188.

Fredholm BB, Ijzerman AP, Jacobso KA, Klotz KN, Linden J (2001). International Union of Pharmacology. XXV. Nomenclature and classification of adenosine receptors. *Pharmacol Rev* **53**: 527–552.

Fredholm BB, Zhang Y, van der Ploeg I (1996). Adenosine A_{2A} receptors mediate the inhibitory effect of adenosine on formyl-Met-Leu-Phe-stimulated respiratory burst in neutrophil leucocytes. *Naunyn Schmiedebergs Arch Pharmacol* **354**: 262–267.

Hakonarson H, Shanbaky I, Guerra FM, Grunstein MM (1998). Modulation of adenosine A_1, bradykinin B_1, and histamine H_1 receptor expression and function in atopic asthmatic sensitized airway smooth muscle. *Am J Respir Crit Care Med* Abstracts issue, C56.

Hannon JP, Bray-French KM, Phillips RM, Fozard JR (1998). Further pharmacological characterization of the adenosine receptor subtype mediating inhibition of oxidative burst in human isolated neutrophils. *Drug Dev Res* **43**: 214–224.

Hasko G, Pacher P, Deitch EA, Vizi ES (2007). Shaping of monocyte and macrophage function by adenosine receptors. *Pharmacol Ther* **113**: 264–275.

Holgate ST (2005). The identification of the adenosine A_{2B} receptor as a novel therapeutic target in asthma. *Br J Pharmacol* **145**: 1009–1015.

Hua X, Erikson CJ, Chason KD, Rosebrock CN, Deshpande DA, Penn RB *et al*. (2007). Involvement of A_1 adenosine receptors and neural pathways in adenosine-induced bronchoconstriction in mice. *Am J Physiol Lung Cell Mol Physiol* **293**: L25–L32.

Huang S, Apasov S, Koshiba M, Sitkovsky M (1997). Role of A_{2a} extracellular adenosine receptor-mediated signaling in adenosine-mediated inhibition of T-cell activation and expansion. *Blood* **90**: 1600–1610.

Hughes PJ, Holgate ST, Church MK (1984). Adenosine inhibits and potentiates IgE-dependent histamine release from human lung mast cells by an A_2-purinoceptor mediated mechanism. *Biochem Pharmacol* **33**: 3847–3852.

Huszar E, Vass G, Vizi E, Csoma Z, Barat E, Molnar Vilagos G *et al*. (2002). Adenosine in exhaled breath condensate in healthy volunteers and in patients with asthma. *Eur Respir J* **20**: 1393–1398.

Jacques CA, Spur BW, Johnson M, Lee TH (1991). The mechanism of LTE4-induced histamine hyperresponsiveness in guinea-pig tracheal and human bronchial smooth muscle, *in vitro*. *Br J Pharmacol* **104**: 859–866.

Jacques CA, Spur BW, Johnson M, Lee TH (1992). The effect of epithelium removal on leukotriene E4-induced histamine hyperresponsiveness in guinea-pig tracheal smooth muscle. *Br J Pharmacol* **106**: 556–562.

Jatakanon A, Uasuf C, Maziak W, Lim S, Chung KF, Barnes PJ (1999). Neutrophilic inflammation in severe persistent asthma. *Am J Respir Crit Care Med* **160** (5 Part 1): 1532–1539.

Joad JP (1990). Characterization of the human peripheral lung adenosine receptor. *Am J Respir Cell Mol Biol* **2**: 193–198.

Johnson HG, McNee ML (1985). Adenosine-induced secretion in the canine trachea: modification by methylxanthines and adenosine derivatives. *Br J Pharmacol* **86**: 63–67.

Keir S, Boswell-Smith V, Spina D, Page C (2006). Mechanism of adenosine-induced airways obstruction in allergic guinea pigs. *Br J Pharmacol* **147**: 720–728.

Knight D, Zheng X, Rocchini C, Jacobson M, Bai T, Walker B (1997). Adenosine A_3 receptor stimulation inhibits migration of human eosinophils. *J Leukoc Biol* **62**: 465–468.

Lappas CM, Sullivan GW, Linden J (2005). Adenosine A_{2A} agonists in development for the treatment of inflammation. *Expert Opin Investig Drugs* **14**: 797–806.

Lee TH, Austen KF, Corey EJ, Drazen JM (1984). Leukotriene E4-induced airway hyperresponsiveness of guinea pig tracheal smooth muscle to histamine and evidence for three separate sulfidopeptide leukotriene receptors. *Proc Natl Acad Sci USA* **81**: 4922–4925.

Lever R, Hefni A, Moffatt JD, Paul W, Page CP (2007). Effect of tecastemizole on pulmonary and cutaneous allergic inflammatory responses. *Clin Exp Allergy* **37**: 909–917.

Liesker JJ, Ten Hacken NH, Rutgers SR, Zeinstra-Smith M, Postma DS, Timens W (2007). Mast cell numbers in airway smooth muscle and PC20AMP in asthma and COPD. *Respir Med* **101**: 882–887.

Luijk B, van den Berge M, Kerstjens A, Postma DD, Lammers JWJ (2003). Effect of an inhaled adenosine A_{2A} agonist on the allergen induced late asthmatic response. *Eur Respir J* **22** (Suppl. 45): 204s.

Mann JS, Holgate ST, Renwick AG, Cushley MJ (1986). Airway effects of purine nucleosides and nucleotides and release with bronchial provocation in asthma. *J Appl Physiol* **61**: 1667–1676.

Marquardt DL, Parker CW, Sullivan TJ (1978). Potentiation of mast cell mediator release by adenosine. *J Immunol* **120**: 871–878.

Marquardt DL, Walker LL, Wasserman SI (1984). Adenosine receptors on mouse bone marrow-derived mast cells: functional significance and regulation by aminophylline. *J Immunol* **133**: 932–937.

McNamara N, Gallup M, Khong A, Sucher A, Maltseva I, Fahy J *et al*. (2004). Adenosine up-regulation of the mucin gene, *MUC2*, in asthma. *FASEB J* **18**: 1770–1772.

Mubagwa K, Mullane K, Flameng W (1996). Role of adenosine in the heart and circulation. *Cardiovasc Res* **32**: 797–813.

Mustafa SJ, Nadeem A, Fan M, Zhong H, Belardinelli L, Zeng D (2007). Effect of a specific and selective A_{2B} adenosine receptor antagonist on adenosine agonist AMP and allergen-induced airway responsiveness and cellular influx in a mouse model of asthma. *J Pharmacol Exp Ther* **320**: 1246–1251.

Nadeem A, Fan M, Ansari HR, Ledent C, Jamal Mustafa S (2007). Enhanced airway reactivity and inflammation in A_{2A} adenosine receptor-deficient allergic mice. *Am J Physiol Lung Cell Mol Physiol* **292**: L1335–L1344.

Nadeem A, Obiefuna PC, Wilson CN, Mustafa SJ (2006). Adenosine A_1 receptor antagonist versus montelukast on airway reactivity and inflammation. *Eur J Pharmacol* **551**: 116–124.

Nelson HS (2003). Prospects for antihistamines in the treatment of asthma. *J Allergy Clin Immunol* **112** (Suppl. 4): S96–S100.

Newby AC (1984). Adenosine and the concept of retaliatory metabolites. *Trends Biochem Sci* **9**: 42–44.

Nightingale JA, Rogers DF, Barnes PJ (1999). Differential effect of formoterol on adenosine monophosphate and histamine reactivity in asthma. *Am J Respir Crit Care Med* **159**: 1786–1790.

Nyce JW (1999). Insight into adenosine receptor function using antisense and gene-knockout approaches. *Trends Pharmacol Sci* **20**: 79–83.

Nyce JW, Metzger WJ (1997). DNA antisense therapy for asthma in an animal model. *Nature* **385**: 721–725.

O'Connor BJ, Chung KF, Chen-Worsdell YM, Fuller RW, Barnes PJ (1991). Effect of inhaled furosemide and bumetanide on adenosine 5′-monophosphate- and sodium metabisulfite-induced bronchoconstriction in asthmatic subjects. *Am Rev Respir Dis* **143**: 1329–1333.

Obiefuna PC, Batra VK, Nadeem A, Borron P, Wilson CN, Mustafa SJ (2005). A novel A_1 adenosine receptor antagonist (L-97-1) reduces allergic responses to house dust mite in an allergic rabbit model of asthma. *J Pharmacol Exp Ther* **315**: 329–336.

Panther E, Idzko M, Herouy Y, Rheinen H, Gebicke-Haerter PJ, Mrowietz U *et al*. (2001). Expression and function of adenosine receptors in human dendritic cells. *FASEB J* **15**: 1963–1970.

Pascoe SJ, Knight H, Woessner R (2007). QAF805, an A_{2B}/A_3 antagonist, does not attenuate AMP challenge in subjects with asthma. *Proceedings of the American Thoracic Society* A682.

Peachell PT, Columbo M, Kagey-Sobotka A, Lichtenstein LM, Marone G (1988). Adenosine potentiates mediator release from human lung mast cells. *Am Rev Respir Dis* **138**: 1143–1151.

Peachell PT, Lichtenstein LM, Schleimer RP (1991). Differential regulation of human basophil and lung mast cell function by adenosine. *J Pharmacol Exp Ther* **256**: 717–726.

Phillips GD, Finnerty JP, Holgate ST (1990). Comparative protective effect of the inhaled beta 2-agonist salbutamol (albuterol) on bronchoconstriction provoked by histamine, methacholine, and adenosine 5′-monophosphate in asthma. *J Allergy Clin Immunol* **85**: 755–762.

Phillips GD, Holgate ST (1989). The effect of oral terfenadine alone and in combination with flurbiprofen on the bronchoconstrictor response to inhaled adenosine 5′-monophosphate in nonatopic asthma. *Am Rev Respir Dis* **139**: 463–469.

Phillips GD, Rafferty P, Beasley R, Holgate ST (1987). Effect of oral terfenadine on the bronchoconstrictor response to inhaled histamine and adenosine 5′-monophosphate in non-atopic asthma. *Thorax* **42**: 939–945.

Phillips GD, Scott VL, Richards R, Holgate ST (1989). Effect of nedocromil sodium and sodium cromoglycate against bronchoconstriction induced by inhaled adenosine 5′-monophosphate. *Eur Respir J* **2**: 210–217.

Polosa R, Ng WH, Crimi N, Vancheri C, Holgate ST, Church MK *et al*. (1995). Release of mast-cell-derived mediators after endobronchial adenosine challenge in asthma. *Am J Respir Crit Care Med* **151** (3 Part 1): 624–629.

Polosa R, Phillips GD, Rajakulasingam K, Holgate ST (1991). The effect of inhaled ipratropium bromide alone and in combination with oral terfenadine on bronchoconstriction provoked by adenosine 5′-monophosphate and histamine in asthma. *J Allergy Clin Immunol* **87**: 939–947.

Polosa R, Rajakulasingam K, Church MK, Holgate ST (1992). Repeated inhalation of bradykinin attenuates adenosine 5′-monophosphate (AMP) induced bronchoconstriction in asthmatic airways. *Eur Respir J* **5**: 700–706.

Polosa R, Rajakulasingam K, Prosperini G, Church MK, Holgate ST (1993). Relative potencies and time course of changes in adenosine 5′-monophosphate airway responsiveness with inhaled furosemide and bumetanide in asthma. *J Allergy Clin Immunol* **92**: 288–297.

Polosa R, Santonocito G, Magrì S, Paolino G, Armato F, Pagano C *et al*. (1997). Neutral endopeptidase inhibition with inhaled phosphoramidon: no effect on bronchial responsiveness to adenosine 5′-monophosphate (AMP) in asthma. *Eur Respir J* **10**: 2460–2464.

Rafferty P, Beasley R, Holgate ST (1987a). The contribution of histamine to immediate bronchoconstriction provoked by inhaled allergen and adenosine 5′ monophosphate in atopic asthma. *Am Rev Respir Dis* **136**: 369–373.

Rafferty P, Beasley R, Southgate P, Holgate S (1987b). The role of histamine in allergen and adenosine-induced bronchoconstriction. *Int Arch Allergy Appl Immunol* **82**: 292–294.

Ralevic V, Burnstock G (1998). Receptors for purines and pyrimidines. *Pharmacol Rev* **50**: 413–492.

Ramkumar V, Stiles GL, Beaven MA, Ali H (1993). The A_3 adenosine receptor is the unique adenosine receptor which facilitates release of allergic mediators in mast cells. *J Biol Chem* **268**: 16887–16890.

Reiss TF, Sorkness CA, Stricker W, Botto A, Busse WW, Kundu S *et al*. (1997). Effects of montelukast (MK-0476); a potent cysteinyl leukotriene receptor antagonist, on bronchodilation in asthmatic subjects treated with and without inhaled corticosteroids. *Thorax* **52**: 45–48.

Riffo-Vasquez Y, Spina D, Page C, Tormay P, Singh M, Henderson B *et al*. (2004). Effect of mycobacterium tuberculosis chaperonins on bronchial eosinophilia and hyper-responsiveness in a murine model of allergic inflammation. *Clin Exp Allergy* **34**: 712–719.

Rorke S, Jennison S, Jeffs JA, Sampson AP, Arshad H, Holgate ST (2002). Role of cysteinyl leukotrienes in adenosine 5′-monophosphate induced bronchoconstriction in asthma. *Thorax* **57**: 323–327.

Ryzhov S, Goldstein AE, Matafonov A, Zeng D, Biaggioni I, Feoktistov I (2004). Adenosine-activated mast cells induce IgE synthesis by B lymphocytes: an A_{2B}-mediated process involving Th2 cytokines IL-4 and IL-13 with implications for asthma. *J Immunol* **172**: 7726–7733.

Salmon JE, Brogle N, Brownlie C, Edberg JC, Kimberly RP, Chen BX *et al*. (1993). Human mononuclear phagocytes express adenosine A1 receptors. A novel mechanism for differential regulation of Fc gamma receptor function. *J Immunol* **151**: 2775–2785.

Spicuzza L, Di Maria G, Polosa R (2006). Adenosine in the airways: implications and applications. *Eur J Pharmacol* **533**: 77–88.

Spicuzza L, Polosa R (2003). The role of adenosine as a novel bronchoprovocant in asthma. *Curr Opin Allergy Clin Immunol* **3**: 65–69.

Spruntulis LM, Broadley KJ (2001). A_3 receptors mediate rapid inflammatory cell influx into the lungs of sensitized guinea-pigs. *Clin Exp Allergy* **31**: 943–951.

Sullivan AK, Uhlson CL, Murphy RC, Voelkel NP (2005). Elevated adenosine in the exhaled air of patients with COPD. *Am J Respir Crit Care Med* Abstracts issue, A653.

Summers QA, Honeywell R, Renwick AG, Holgate ST (1990). The protective efficacy of inhaled, oral and intravenous nedocromil sodium against adenosine-5′-monophosphate-induced bronchoconstriction in asthmatic volunteers. *Pulm Pharmacol* **3**: 190–197.

Sun CX, Young HW, Molina JG, Volmer JB, Schnermann J, Blackburn MR (2005). A protective role for the A_1 adenosine receptor in adenosine-dependent pulmonary injury. *J Clin Invest* **115**: 35–43.

Suzuki H, Takei M, Nakahata T, Fukamachi H (1998). Inhibitory effect of adenosine on degranulation of human cultured mast cells upon cross-linking of Fc epsilon RI. *Biochem Biophys Res Commun* **242**: 697–702.

Tilley SL, Tsai M, Williams CM, Wang ZS, Erikson CJ, Galli SJ *et al*. (2003). Identification of A_3 receptor- and mast cell-dependent and -independent components of adenosine-mediated airway responsiveness in mice. *J Immunol* **171**: 331–337.

van den Berge M, Kerstjens HA, de Reus DM, Koeter GH, Kauffman HF, Postma DS (2004). Provocation with adenosine 5′-monophosphate, but not methacholine, induces sputum eosinophilia. *Clin Exp Allergy* **34**: 71–76.

Varani K, Caramori G, Vincenzi F, Adcock I, Casolari P, Leung E *et al*. (2006). Alteration of adenosine receptors in patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* **173**: 398–406.

Vizi E, Huszar E, Csoma Z, Boszormenyi-Nagy G, Barat E, Horvath I *et al*. (2002). Plasma adenosine concentration increases during exercise: a possible contributing factor in exercise-induced bronchoconstriction in asthma. *J Allergy Clin Immunol* **109**: 446–448.

Walker BA, Jacobson MA, Knight DA, Salvatore CA, Weir T, Zhou D *et al*. (1997). Adenosine A_3 receptor expression and function in eosinophils. *Am J Respir Cell Mol Biol* **16**: 531–537.

Walsh GM (2002). Second-generation antihistamines in asthma therapy: is there a protective effect? *Am J Respir Med* **1**: 27–34.

Wilson CN, Batra VK (2002). Lipopolysaccharide binds to and activates A_1 adenosine receptors on human pulmonary artery endothelial cells. *J Endotoxin Res* **8**: 263–271.

Wollner A, Wollner S, Smith JB (1993). Acting via A_2 receptors, adenosine inhibits the upregulation of Mac-1 (Cd11b/CD18) expression on FMLP-stimulated neutrophils. *Am J Respir Cell Mol Biol* **9**: 179–185.

Wood-Baker R, Holgate ST (1993). The comparative actions and adverse effect profile of single doses of H_1-receptor antihistamines in the airways and skin of subjects with asthma. *J Allergy Clin Immunol* **91**: 1005–1014.

Young HW, Molina JG, Dimina D, Zhong H, Jacobson M, Chan LN *et al*. (2004). A_3 adenosine receptor signaling contributes to airway inflammation and mucus production in adenosine deaminase-deficient mice. *J Immunol* **173**: 1380–1389.

Zhang JG, Hepburn L, Cruz G, Borman RA, Clark KL (2005). The role of adenosine A_{2A} and A_{2B} receptors in the regulation of TNF-alpha production by human monocytes. *Biochem Pharmacol* **69**: 883–889.

Zhong H, Belardinelli L, Maa T, Feoktisto I, Biaggioni I, Zeng D (2004). A(2B) adenosine receptors increase cytokine release by bronchial smooth muscle cells. *Am J Respir Cell Mol Biol* **30**: 118–125.

Zhong H, Belardinelli L, Maa T, Zeng D (2005). Synergy between A_{2B} adenosine receptors and hypoxia in activating human lung fibroblasts. *Am J Respir Cell Mol Biol* **32**: 2–8.

Zhong H, Chunn JL, Volmer JB, Fozard JR, Blackburn MR (2001). Adenosine-mediated mast cell degranulation in adenosine deaminase-deficient mice. *J Pharmacol Exp Ther* **298**: 433–440.

Zhong H, Wu Y, Belardinelli L, Zeng D (2006). A_{2B} adenosine receptors induce IL-19 from bronchial epithelial cells, resulting in TNF-alpha increase. *Am J Respir Cell Mol Biol* **35**: 587–592.