British Journal of Pharmacology (2005) 145, 1009–1015
www.nature.com/bjp

THE QUINTILES PRIZE LECTURE 2004

The identification of the adenosine A_{2B} receptor as a novel therapeutic target in asthma

*,[1]Stephen T. Holgate

[1]Allergy and Inflammation Research, School of Medicine, University of Southampton, Southampton

Adenosine is a powerful bronchoconstrictor of asthmatic, but not normal, airways. *In vitro* studies on isolated human mast cells and basophils revealed that adenosine and selective analogues augmented inflammatory mediator release from mast cells by stimulating A_2 receptors. Pharmacological blockade of mast cell mediator release *in vivo* also attenuated adenosine-induced bronchoconstriction, as did theophylline, by adenosine A_2 receptor antagonism. Further *in vitro* studies revealed that the asthmatic response to adenosine is likely to be mediated *via* the A_{2B} subtype which is selectively antagonised by enprofylline. Studies in animal models, especially mice, have shown a close synergistic interaction between adenosine, Th2 and airway remodelling responses. The recent description of A_{2B} receptors on human airway smooth muscle cells that mediate cytokine and chemokine release and induce differentiation of fibroblasts into myofibroblasts strengthens the view that adenosine maybe more than an inflammatory mediator in asthma but also participates in airway wall remodelling in this disease. These data have provided a firm basis for developing adenosine A_{2B} receptor antagonists as a new therapeutic approach to this disease.
British Journal of Pharmacology (2005) **145,** 1009–1015. doi:10.1038/sj.bjp.0706272;
published online 27 June 2005

Keywords: Adenosine; asthma; bronchoconstriction; xanthines; new therapies

Abbreviations: 3′5′-AMP, 3′5′-cyclic adenosine monophosphate; AMP, adenosine 5′-monophosphate; ATP, adenosine triphosphate; IPDX, 3-isobutyl-8-pyrrolidinoxanlthine; LTC_4, leukotriene C_4; NECA, 5′-*N*-ethylcarboximidoadenosine; PGD_2, prostaglandin D_2; UTP, uridine triphosphate

Early observations

Adenosine is a purine nucleoside that plays a key role in nucleic acid, energy and protein metabolism. As an extracellular autacoid generated by 5′-nucleoside cleavage of adenosine 5′-monophosphate, it is a powerful mediator acting through specific cell surface purinoreceptors. In 1978, while working as a post-doctoral research fellow in Dr Frank Austen's laboratory at Harvard University, I showed that adenosine and related synthetic analogues were potent agents in augmenting IgE-dependent mediator release from isolated rodent mast cells (Holgate *et al*., 1980). On returning to the UK in 1980, I set about exploring whether adenosine had any role as a mediator of asthma. In 1983, Michael Cushley, a clinical research fellow, demonstrated that inhaled adenosine (but not its metabolite inosine or the unrelated nucleoside guanosine) was a powerful bronchoconstrictor of asthmatic but, importantly, not of normal airways (Cushley *et al*., 1983b). Further work showed that both allergic and non-allergic asthmatics responded in a similar way and that the effect was also seen with adenosine 5′-monophosphate (AMP) and adenosine 5′-diphosphate (ADP) (Mann *et al*., 1986b). A detectable, but lesser response of the lower airways was also observed in patients with allergic rhinitis (Phillips *et al*., 1990). However, when adenosine was injected intradermally into atopic skin, the vasodilator and small wheal response was no different from that observed in nonatopic skin (Djukanovic *et al*., 1989). Since asthma accompanies rhinitis in ~80% of patients, the intermediate airway response observed with adenosine challenge in allergic rhinitis was most likely due to concomitant mild asthma (Djukanovic *et al*., 1992; Doull *et al*., 1996), but could not be explained by a generalised increased responsiveness of epithelial surfaces in atopic subjects. As AMP was more soluble in an aqueous solvent than adenosine, most of the future inhalation challenge work was conducted using this nucleotide.

These preliminary observations led to the hypothesis that *'adenosine, which accumulates in inflamed mucosa under conditions of cell stress and hypoxia, contributes as a mediator of bronchoconstriction in both acute and chronic asthma'*. To pursue this, we first demonstrated that following inhalation allergen challenge of sensitised asthmatic subjects adenosine was released into the circulation (Mann *et al*., 1986a) and locally into the airways (Polosa *et al*., 1995). Adenosine was also shown to be released from antigen-challenged human lung fragments *in vitro* in the presence of inhibitors of adenosine deaminase and adenosine kinase (Konnaris & Lloyd, 1996). Blockade of adenosine re-uptake by dipyridamole increased the bronchoconstrictor response to inhaled AMP, indicating that accumulation of extracellular adenosine was closely associated with the asthmatic airway response (Cushley *et al*., 1985). The ability of dipyridamole to enhance another adenosine-mediated effect was later shown in humans on the hypercapnic ventilatory response, thereby confirming its mode

*Author for correspondence at: Allergy & Inflammation Research, Level F, South Academic Block (810), Southampton General Hospital, Southampton SO16 6YD; E-mail: S.Holgate@soton.ac.uk

of action *in vivo* of increasing extracellular adenosine levels (Griffiths *et al*., 1990; 1997). *In vitro* studies confirmed that adenosine and A_2 receptor analogues (e.g. 5′-*N*-ethylcarboxamidoadenosine (NECA)) could augment IgE-dependent mediator release from both human mast cells and basophils (Church *et al*., 1983; Hughes *et al*., 1983; 1984) and that activated leukocytes were a major source of extracellular adenosine (Mann *et al*., 1986c). Adenosine also releases histamine directly from human bronchoalveolar lavage mast cells (Forsythe *et al*., 1999).

Mechanism(s) of adenosine-induced bronchoconstriction

The possibility that adenosine caused bronchoconstriction in asthma indirectly *via* mast cell activation as suggested by our early *in vitro* studies was pursued in several ways. Firstly, AMP provocation of asthmatic airways *in vivo* was accompanied by a rise in circulating histamine levels (Phillips *et al*., 1990). Secondly, the immediate bronchoconstriction provoked by inhaled AMP was shown to be antagonised by inhibiting the effects of individual mast cell mediators using selective histamine H_1 antagonists (e.g. terfenadine, astemizole) (Holgate, 1987; Phillips *et al*., 1987; Rafferty *et al*., 1987a, b; Phillips & Holgate, 1989), cysteinyl leukotriene receptor 1 (cysLT_1) antagonists (e.g. montelukast) (Rorke *et al*., 2002) and inhibition of cyclooxygenase 1 and 2 (e.g. flurbiprofen and indomethacin) (Crimi *et al*., 1989; Phillips & Holgate, 1989). Inhibition of cyclooxygenase activity ablates the production of the powerful bronchoconstrictor mediator prostaglandin (PG)D_2 from activated mast cells. Secondly, the mast cell stabilising drugs sodium cromoglicate (Richards *et al*., 1988; Phillips *et al*., 1989b; Richards *et al*., 1989), nedocromil sodium (Phillips *et al*., 1989b; Richards *et al*., 1989; Summers *et al*., 1990; Church & Holgate, 1993) and more recently andolast (Persiani *et al*., 2001) were shown to be powerful inhibitors of AMP-induced bronchoconstriction in asthma. Thirdly, when administered by inhalation, the loop diuretics frusemide and bumetanide also inhibited adenosine-provoked bronchoconstriction through their known inhibitory effects in ion channels on mast cells to reduce their threshold of activation and mediator secretion (Polosa *et al*., 1993a; Rajakulasingam *et al*., 1994; Bradding *et al*., 2003; Duffy *et al*., 2004). Heparin, a highly sulphated unbranched glycosaminoglycan, when given by inhalation protects against bronchoconstriction provoked by allergen and exercise (Ahmed *et al*., 1993; Bowler *et al*., 1993) is also inhibitory against AMP challenge of the lower (Polosa *et al*., 1997) and upper (Zang *et al*., 2004) airways, again through suppression of mast cell mediator release.

In allergic asthmatics, AMP-induced bronchoconstriction with inhaled AMP was more rapid in onset than that observed with inhaled allergen, indicating that airway narrowing was the consequence of rapid mast cell degranulation with release of histamine and generation of newly formed eicosanoids – PGD_2 and LTC_4 (Cushley & Holgate, 1985; Phillips & Holgate, 1988a) rather than the additional induction of newly formed cytokines and chemokines that are considered to underpin the late phase allergen response (Phillips & Holgate, 1988b). The absence of a late-phase response with inhaled AMP provocation highlighted a fundamental difference in the way that adenosine and allergen interacted with airway mast cells for mediator secretion (Holgate *et al*., 1987; Church & Holgate, 1988; Holgate *et al*., 1988). Blockade of muscarinic cholinergic receptors using inhaled ipratropium bromide had only minimal effect in antagonising bronchoconstriction provoked by AMP, leading to the conclusion that cholinergic reflexes were of limited importance in mediating bronchoconstriction (Mann *et al*., 1985; Polosa *et al*., 1991). By contrast, inhaled β_2-agonists such as salbutamol had a powerful inhibitory effect on AMP-induced bronchoconstriction by serving as a functional antagonist and as a direct inhibitor of human mast cell activation–secretion coupling (Phillips *et al*., 1990a).

Unusual features of adenosine-induced bronchoconstriction

Several interesting features about the pro-asthmatic effect of adenosine have emerged. Repeated provocation of asthmatic airways with inhaled AMP led to the development of tolerance, which took 6–8 h to recover (Daxun *et al*., 1989). Of significance was the further finding that, while in this refractory state, the airways were hyperresponsive to allergen inhalation, suggesting that prior adenosine exposure had produced mast cell priming as we had previously demonstrated *in vitro* (Holgate *et al*., 1980; Church *et al*., 1983; Hughes *et al*., 1983; 1984; Phillips *et al*., 1989a). Bronchoconstriction provoked by AMP also rendered the airways refractory to exercise and inhaled bradykinin and *vice versa*, but not to methacholine challenge, suggesting that the former stimuli operated through a common mast cell-mediated mechanism (Finnerty *et al*., 1990; Polosa *et al*., 1992). It has long been known that exercise-induced asthma is a mast cell-dependent phenomena (Finnerty & Holgate, 1990; 1993; Roach *et al*., 1998), but cross-tolerance between AMP and bradykinin was less easy to explain. We had shown that bradykinin caused bronchoconstriction through activation of bradykinin B2 receptors (Polosa & Holgate, 1990) and that repeated challenge with this peptide also rapidly led to the development of tolerance (Polosa *et al*., 1993b; Rajakulasingam *et al*., 1993). Both bradykinin B2 and adenosine receptors have been identified on mast cells (Reissmann *et al*., 2000; Sylvin *et al*., 2001) and also on peptidergic nerves (Fox *et al*., 1996; Chung, 2002), raising the possibility that adenosine and bradykinin share some common activation pathways possibly through the release of neuropeptides such as substance P or other neurokinins, which are known to activate mast cells for mediator secretion (Rajakulasingam *et al*., 1994).

Adenosine receptors mediate the proasthmatic response

Early work on both rodent and human mast cells demonstrated that adenosine was a powerful stimulator of mast cell and basophil adenylate cyclase to increase cellular levels of cyclic 3′5′-AMP operating through the A_2 subtype of purinoceptor (Holgate *et al*., 1980; Hughes *et al*., 1983; 1984; Church & Holgate, 1993). Shortly after describing the bronchoconstrictor activity of adenosine, we demonstrated that both inhaled (Cushley *et al*., 1983a; 1984; Holgate *et al*., 1984) and oral theophylline were able to selectively antagonise AMP-induced bronchoconstriction beyond their ability to act

as functional antagonists (Mann & Holgate, 1985; Church *et al*., 1986). The fact that this occurred at drug concentrations one order of magnitude lower than that required to inhibit cyclic AMP phosphodiesterase and in the same range as therapeutic plasma concentrations of theophylline (Holgate *et al*., 1987) opened up the possibility that the known antiasthmatic effect of methylxanthines could, in part, be due to adenosine antagonism. This view was challenged when enprofylline became available because it was shown that this drug was a powerful inhibitor of cyclic AMP phosphodiesterase but, different from theophylline, was devoid of A_2 receeptor antagonism that had been linked to the diuretic and cardiac arrhythmic properties of xanthines (Lunell *et al*., 1983; Persson *et al*., 1986). Thus, in the early 1990s, the idea that adenosine was an important mediator of asthma was being seriously eroded.

However, based on current *in vitro* pharmacology available at the time, it had been assumed that adenosine was active through a single A_2 receptor linked to adenylate cyclase and that was quite distinct from the other purinergic receptors that responded more selectively to ATP and UTP (e.g. P2Yand P2X). However, a paradox that could not be explained was how an agent which increased cyclic AMP within mast cells and basophils could augment rather than inhibit mediator release, as would be expected since increases in cyclic 3′5′-AMP produced by other agonists, for example, with β_2-adrenoceptor agonists (Okayama & Church, 1992) or PG E_2 (Peters *et al*., 1982) were strongly inhibitory for mediator release. Further clarity came with the discovery that adenosine A_2 receptors existed as two subtypes – A_{2A} linked to adenylate cyclase and involving G_s coupling, and A_{2B} linked to both adenylate cyclase and the phosphatidyl trisphosphate (PI_3)-calcium signalling pathway involving both G_s and G_q coupling (Feoktistov & Biaggioni, 1995; Feoktistov *et al*., 1998). Thus, while exhibiting no antagonist properties against adenosine A_{2A} receptors, enprofylline was shown to be a highly selective, albeit weak, antagonist of A_{2B} receptors (Feoktistov & Biaggioni, 1995; Kim *et al*., 2002; Fan *et al*., 2003). This critical observation helped explain our finding of a preferential inhibitory effect of intravenous emprofylline on AMP-induced bronchoconstriction (Clarke *et al*., 1989). The identification of the A_{2B} receptor subtype revitalised interest in adenosine as a mediator of asthma and becoming a new therapeutic target for this disease (Feoktistov *et al*., 1998). Although most of the work identifying A_{2B} receptors on human mast cells was conducted on the HMC-1 mastocytosis derived cell line, recently A_{2B} receptors mediating enhanced mediator release have also been found on mast cells dispersed from human lung tissue (Zhong H, personal communication). In addition to causing mast cell mediator release, activation of A_{2B} receptors on HMC-1 cells cultured with human B cells results in Ig isotype, switching to IgE involving costimulation utilising CD40 and enhanced IL-4 and IL-13 secretion (Ryzhov *et al*., 2004).

With the identification of this new subclass of A_2 receptors, the ease with which repeated exposure to adenosine (and AMP) results in tolerance and cross-tolerance became of the target of further study. The A_{2B} receptor appears to be regulated differently from many other G-protein-coupled receptors. Mundell and co-workers have shown that agonist activation of A_{2B} receptors results in arrestin-dependent internalisation of the receptor complex with antisense neutralisation of arrestin, resulting in loss of desensitisation (Mundell *et al*., 2000; Matharu *et al*., 2001). Recent work has shown that human A_{2B} receptors associate with intracellular signalling proteins other than G proteins such as those containing PDZ (PSD-95, Dig 20-1) domains, and more specifically with the PDZ domain-containing protein E3KARP (Sitaraman *et al*., 2002). This is known to interact with ezrin/radixin/moesin (ERM) proteins which in turn interact with the actin cytoskeleton that control A_{2B} receptor trafficking. This molecular-based work provides a good explanation for the ease with which A_{2B} receptor stimulation results in rapid and profound tachyphylaxis, and also for cross-desensitisation between A_{2B} and other G-protein-coupled receptors (Sitaraman *et al*., 2000).

The first observation that inhaled corticosteroids were highly active in rapidly suppressing AMP-induced bronchoconstriction (Doull *et al*., 1997; Holgate *et al*., 2000) and the recent demonstration that AMP challenge induces eosinophil influx into the airways (van den Berge *et al*., 2004) further strengthened interest of the role of A_{2B} receptor in asthma. The rapidity with which this occurs (Wilson *et al*., 2003) suggests that a unique effect of corticosteroids on the A_{2B} receptor internalisation mechanisms possibly involving the recently described rapid steroid response receptor (Long *et al*., 2005).

Observation on the role of adenosine in animal models

Adenosine receptors are also involved in mediating bronchoconstriction in a number of animal models, but between animal species there is heterogeneity of the receptors involved. In the rabbit the airway response is mediated through A_1 receptors (Nyce & Metzger, 1997), in the rat by A_1, A_{2B} and A_3 receptors (Pauwels & Van der Straeten, 1987) or an atyptical adenosine receptor (Hannon *et al*., 2002), in the guinea-pig by A_3 receptors (Thorne *et al*., 1996) and in the mouse by A_{2B} and A_3 receptors (Fan *et al*., 2003). It has further been shown that adenosine deaminase (ADA)-deficient mice develop progressive lung inflammation which can be effectively reversed by adenosine deaminase therapy and markedly reduced by treatment with selective adenosine A_{2B} receptor antagonists (Chunn *et al*., 2001). Using mice lacking the A_{2A} receptor and, therefore, the adenylate cyclase signal associated with its activation (Ohta & Sitkovsky, 2001), a key role for endogenously generated adenosine in providing a regulatory feedback mechanism capable of limiting or terminating inflammatory responses has been shown. In a rat 'model' of allergic asthma, the A_{2A} agonist CGS 21680 exhibits anti-inflammatory activity similar to that of the corticosteroid, budesonide (Fozard *et al*., 2002). Most recently, Sun *et al*. (2005) have shown that the A_1 receptor plays an anti-inflammatory role in the pulmonary phenotype seen in ADA-deficient mice. GlaxoSmithKline are also investigating an inhaled A_{2A} agonist GW328267X in both asthma and chronic obstructive pulmonary disease (Luijk *et al*., 2003), but this has recently been dropped from development due to cardiovascular side effects. On the proinflammatory side, the important influence that adenosine has over asthma pathogenesis has recently received additional support from the observation that, in dual transgenic mice, adenosine and the pro-inflammatory and pro-remodelling cytokine interluekin-13 interact synergistically (Blackburn

et al., 2003). Since there is now good evidence to support the involvement of A_{2B} receptor in mast cell activation, promising antagonists for this receptor are being developed, such as IPDX (Feoktistov *et al*., 2001), 8-SPT, XAC, CGS15493 (Fozard *et al*., 2003) and CVT 6883. Some of these are now entering clinical trial in the long-term treatment of asthma (Fozard, 2003; Wolber & Fozard, 2005).

Adenosine bronchoprovocation as a diagnostic test

A second development from the adenosine research describes in this brief review is the use of adenosine (or AMP) inhalation challenge as a diagnostic test for asthma where its specificity and sensitivity appear to be superior to that of inhaled histamine and methacholine (Holgate, 2002a; Polosa *et al*., 2002; Joos *et al*., 2003). When compared to agents that produce bronchconstriction directly such as methacholine, airway responsiveness to AMP also seems to be more closely associated with airway inflammation (Van den Berge *et al*., 2001). In addition, AMP responsiveness is also used as a test for distinguishing asthma from COPD (Spicuzza *et al*., 2003). Since the airway response to AMP is so sensitive to the effect of inhaled corticosteroids and also is a good marker of disease activity, AMP bronchoprovocation has been suggested as useful as a biomarker to assess disease control (Lee *et al*., 2003; Prieto *et al*., 2003).

Concluding comments

Over a span of 20 years, the initial observation of the unique pro-asthmatic effects of inhaled adenosine has evolved to provide the basis for a new asthma therapy as well as a possible diagnostic test (Holgate, 2002b; Rorke & Holgate, 2002). Recently, Inbe *et al*. (2004) has described a second novel receptor P2Y-15 for adenosine and AMP on human mast cells, but this has recently been challenged (Abbracchio *et al*., 2005). The recent discovery that A_{2B} receptors are also functionally active on human airway smooth muscle cells to enhance cytokine and chemokine release (Zhong *et al*., 2004a, b) and on lung fibroblasts where they promote differentiation to a myofibroblast phenotype (Zhong *et al*., 2004b) adds to the view that this receptor may be involved in airway wall remodelling as well as in inflammation in asthma. The next 5 years will be critical in determining whether targeting the A_{2B} receptor will translate into clinical efficacy for patients with chronic asthma.

I am especially grateful to Professors Martin K. Church and Andrew G. Renwick, for their support over the years to help in the pursuance of this work, and Professor Anne Tattersfield, Dr Michael Cushley, Jonathan Mann, Gerrard Phillips, Ricardo Polosa, Neil Tallant, Tim Griffiths, Iola Doull, Ratko Djukanovic, Paul Rafferty, Phillip Hughes, Steuart Rorke, Robert Richards, James Finnerty and Zhu Daxun, who all contributed to this endeavour, and the Medical Research Council and a number of pharmaceutical companies, who helped fund the studies.

References

ABBRACCHIO, M.P., BURNSTOCK, G., BOEYNAEMS, J.M., BARNARD, E.A., BOYER, J.L., KENNEDY, C., MIRAS-PORTUGAL, M.T., KING, B.F., GACHET, C., JACOBSON, K.A. & WEISMAN, G.A. (2005). The recently deorphanized GPR80 (GPR99) proposed to be the P2Y15 receptor is not a genuine P2Y receptor. *Trends Pharmacol. Sci.,* **26,** 8–9.

AHMED, T., GARRIGO, J. & DANTA, I. (1993). Preventing bronchoconstriction in exercise-induced asthma with inhaled heparin. *N. Engl. J. Med.,* **329,** 90–95.

BLACKBURN, M.R., LEE, C.G., YOUNG, H.W., ZHU, Z., CHUNN, J.L., KANG, M.J., BANERJEE, S.K. & ELIAS, J.A. (2003). Adenosine mediates IL-13-induced inflammation and remodeling in the lung and interacts in an IL-13-adenosine amplification pathway. *J. Clin. Invest.,* **112,** 332–344.

BOWLER, S.D., SMITH, S.M. & LAVERCOMBE, P.S. (1993). Heparin inhibits the immediate response to antigen in the skin and lungs of allergic subjects. *Am. Rev. Respir. Dis.,* **147,** 160–163.

BRADDING, P., OKAYAMA, Y., KAMBE, N. & SAITO, H. (2003). Ion channel gene expression in human lung, skin, and cord blood-derived mast cells. *J. Leukoc. Biol.,* **73,** 614–620.

CHUNG, K.F. (2002). Cough: potential pharmacological developments. *Expert Opin. Investig. Drugs,* **11,** 955–963.

CHUNN, J.L., YOUNG, H.W., BANERJEE, S.K., COLASURDO, G.N. & BLACKBURN, M.R. (2001). Adenosine-dependent airway inflammation and hyperresponsiveness in partially adenosine deaminase-deficient mice. *J. Immunol.,* **167,** 4676–4685.

CHURCH, M.K. & HOLGATE, S.T. (1988). Adenosine in asthmatic lung. *Prog. Clin. Biol. Res.,* **263,** 159–166.

CHURCH, M.K. & HOLGATE, S.T. (1993). Adenosine-induced bronchoconstriction and its inhibition by nedocromil sodium. *J. Allergy Clin. Immunol.,* **92,** 190–194.

CHURCH, M.K., FEATHERSTONE, R.L., CUSHLEY, M.J., MANN, J.S. & HOLGATE, S.T. (1986). Relationships between adenosine, cyclic nucleotides, and xanthines in asthma. *J. Allergy Clin. Immunol.,* **78,** 670–675.

CHURCH, M.K., HOLGATE, S.T. & HUGHES, P.J. (1983). Adenosine inhibits and potentiates IgE-dependent histamine release from human basophils by an A2-receptor mediated mechanism. *Br. J. Pharmacol.,* **80,** 719–726.

CLARKE, H., CUSHLEY, M.J., PERSSON, C.G. & HOLGATE, S.T. (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. *Pulmon. Pharmacol.,* **2,** 147–154.

CRIMI, N., PALERMO, F., POLOSA, R., OLIVERI, R., MACCARRONE, C., PALERMO, B. & MISTRETTA, A. (1989). Effect of indomethacin on adenosine-induced bronchoconstriction. *J. Allergy Clin. Immunol.,* **83,** 921–925.

CUSHLEY, M.J. & HOLGATE, S.T. (1985). Adenosine-induced bronchoconstriction in asthma: role of mast cell-mediator release. *J. Allergy Clin. Immunol.,* **75,** 272–278.

CUSHLEY, M.J., TALLANT, N. & HOLGATE, S.T. (1985). The effect of dipyridamole on histamine- and adenosine-induced bronchoconstriction in normal and asthmatic subjects. *Eur. J Respir. Dis.,* **67,** 185–192.

CUSHLEY, M.J., TATTERSFIELD, A.E. & HOLGATE, S.T. (1983a). Adenosine antagonism as an alternative mechanism of action of methylxanthines in asthma. *Agents Actions Suppl.,* **13,** 109–113.

CUSHLEY, M.J., TATTERSFIELD, A.E. & HOLGATE, S.T. (1983b). Inhaled adenosine and guanosine on airway resistance in normal and asthmatic subjects. *Br. J. Clin. Pharmacol.,* **15,** 161–165.

CUSHLEY, M.J., TATTERSFIELD, A.E. & HOLGATE, S.T. (1984). Adenosine-induced bronchoconstriction in asthma. Antagonism by inhaled theophylline. *Am. Rev. Respir. Dis.,* **129,** 380–384.

DAXUN, Z., RAFFERTY, P., RICHARDS, R., SUMMERELL, S. & HOLGATE, S.T. (1989). Airway refractoriness to adenosine 5′-monophosphate after repeated inhalation. *J. Allergy Clin. Immunol.,* **83,** 152–158.

DJUKANOVIC, R., FINNERTY, J.P. & HOLGATE, S.T. (1989). Wheal-and-flare responses to intradermally injected adenosine 5′-monophosphate, hypertonic saline, and histamine: comparison of atopic and nonatopic subjects. *J. Allergy Clin. Immunol.,* **84,** 373–378.

DJUKANOVIC, R., LAI, C.K., WILSON, J.W., BRITTEN, K.M., WILSON, S.J., ROCHE, W.R., HOWARTH, P.H. & HOLGATE, S.T. (1992). Bronchial mucosal manifestations of atopy: a comparison of markers of inflammation between atopic asthmatics, atopic non-asthmatics and healthy controls. *Eur. Respir J.,* **5,** 538–544.

DOULL, I.J., LAWRENCE, S., WATSON, M., BEGISHVILI, T., BEASLEY, R.W., LAMPE, F., HOLGATE, T. & MORTON, N.E. (1996). Allelic association of gene markers on chromosomes 5q and 11q with atopy and bronchial hyperresponsiveness. *Am. J. Respir. Crit. Care Med.,* **153,** 1280–1284.

DOULL, J., SANDALL, D., SMITH, S., SCHREIBER, J., FREEZER, N.J. & HOLGATE, S.T. (1997). Differential inhibitory effect of regular inhaled corticosteroid on airway responsiveness to adenosine 5′ monophosphate, methacholine, and bradykinin in symptomatic children with recurrent wheeze. *Pediatr. Pulmonol.,* **23,** 404–411.

DUFFY, M.S., BERGER, P., CRUSE, G., YANG, W. & BOLTON, S.J. (2004). The K^+ channel iKCA1 potentiates Ca^{2+} influx and degranulation in human lung mast cells. *J. Allergy Clin. Immunol.,* **114,** 66–72.

FAN, M., QIN, W. & MUSTAFA, S.J. (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.

FEOKTISTOV, I. & BIAGGIONI, I. (1995). Adenosine A2b receptors evoke interleukin-8 secretion in human mast cells. An enprofylline-sensitive mechanism with implications for asthma. *J. Clin. Invest.,* **96,** 1979–1986.

FEOKTISTOV, I., GARLAND, E.M., GOLDSTEIN, A.E., ZENG, D., BELARDINELLI, L., WELLS, J.N. & BIAGGIONI, I. (2001). Inhibition of human mast cell activation with the novel selective adenosine A(2B) receptor antagonist 3-isobutyl-8-pyrrolidinoxanthine (IPDX)(2). *Biochem. Pharmacol.,* **62,** 1163–1173.

FEOKTISTOV, I., POLOSA, R., HOLGATE, S.T. & BIAGGIONI, I. (1998). Adenosine A2B receptors: a novel therapeutic target in asthma? *Trends Pharmacol. Sci.,* **19,** 148–153.

FINNERTY, J.P. & HOLGATE, S.T. (1990). Evidence for the roles of histamine and prostaglandins as mediators in exercise-induced asthma: the inhibitory effect of terfenadine and flurbiprofen alone and in combination. *Eur. Respir. J.,* **3,** 540–547.

FINNERTY, J.P. & HOLGATE, S.T. (1993). The contribution of histamine release and vagal reflexes, alone and in combination, to exercise-induced asthma. *Eur. Respir. J.,* **6,** 1132–1137.

FINNERTY, J.P., POLOSA, R. & HOLGATE, S.T. (1990). Repeated exposure of asthmatic airways to inhaled adenosine 5′-monophosphate attenuates bronchoconstriction provoked by exercise. *J. Allergy Clin. Immunol.,* **86,** 353–359.

FORSYTHE, P., MC GARVEY, L.P.A., HEANEY, L.G., MAC MAHON, J. & ENNIS, M. (1999). Adenosine induces histamine release from human bronchoalveolar lavage mast cells. *Clin. Sci.,* **96,** 349–355.

FOX, A.J., LALLOO, U.G., BELVISI, M.G., BERNAREGGI, M., CHUNG, K.F. & BARNES, P.J. (1996). Bradykinin-evoked sensitization of airway sensory nerves: a mechanism for ACE-inhibitor cough. *Nat. Med.,* **2,** 814–817.

FOZARD, J.R. (2003). The case for a role for adenosine in asthma: almost convincing? *Curr. Opin. Pharmacol.,* **3,** 264–269.

FOZARD, J.R., BAUR, F. & WOLBER, C. (2003). Antagonist pharmacology of adenosine A2B receptors from rat, guinea pig and dog. *Eur. J. Pharmacol.,* **475,** 79–84.

FOZARD, J.R., ELLIS, K.M., VILLELA DANTAS, M.F., TIGANI, B. & MAZZONI, L. (2002). Effects of CGS 21680, a selective adenosine A2A receptor agonist, on allergic airways inflammation in the rat. *Eur. J. Pharmacol.,* **438,** 183–188.

GRIFFITHS, T.L., CHRISTIE, J.M., PARSONS, S.T. & HOLGATE, S.T. (1997). The effect of dipyridamole and theophylline on hypercapnic ventilatory responses: the role of adenosine. *Eur. Respir. J.,* **10,** 156–160.

GRIFFITHS, T.L., WARREN, S.J., CHANT, A.D. & HOLGATE, S.T. (1990). Ventilatory effects of hypoxia and adenosine infusion in patients after bilateral carotid endarterectomy. *Clin. Sci. (London),* **78,** 25–31.

HANNON, J.P., TIGANI, B., WOLBER, C., WILLIAMS, I., MAZZANI, L., HOWES, C. & FOZZARD, J.R. (2002). Evidence from atypical receptor mediating the augmented bronchoconstrictor response to adenosine induced by allergen challenge in actively sensitised Brown Norway rats. *Br. J. Pharmacol.,* **135,** 685–696.

HOLGATE, S.T. (1987). Contribution of inflammatory mediators to the immediate asthmatic reaction. *Am. Rev. Respir. Dis.,* **135,** S57–S62.

HOLGATE, S.T. (2002a). Adenosine provocation: a new test for allergic type airway inflammation. *Am. J. Respir. Crit. Care Med.,* **165,** 317–318.

HOLGATE, S.T. (2002b). Adenosine: a key effector molecule of asthma or just another mediator? *Am. J. Physiol. Lung Cell. Mol. Physiol.,* **282,** L167–L168.

HOLGATE, S.T., ARSHAD, H., STRYSZAK, P. & HARRISON, J.E. (2000). Mometasone furoate antagonizes AMP-induced bronchoconstriction in patients with mild asthma. *J. Allergy Clin. Immunol.,* **105,** 906–911.

HOLGATE, S.T., LEWIS, R.A. & AUSTEN, K.F. (1980). Role of adenylate cyclase in immunologic release of mediators from rat mast cells: agonist and antagonist effects of purine- and ribose-modified adenosine analogs. *Proc. Natl. Acad. Sci. U.S.A.,* **77,** 6800–6804.

HOLGATE, S.T., MANN, J.S., CHURCH, M.K. & CUSHLEY, M.J. (1987). Mechanisms and significance of adenosine-induced bronchoconstriction in asthma. *Allergy,* **42,** 481–484.

HOLGATE, S.T., MANN, J.S. & CUSHLEY, M.J. (1984). Adenosine as a bronchoconstrictor mediator in asthma and its antagonism by methylxanthines. *J. Allergy Clin. Immunol.,* **74,** 302–306.

HOLGATE, S.T., ROBINSON, C. & CHURCH, M.K. (1988). The contribution of mast cell mediators to acute allergic reactions in human skin and airways. *Allergy,* **43** (Suppl 5), 22–31.

HUGHES, P.J., HOLGATE, S.T. & CHURCH, M.K. (1984). Adenosine inhibits and potentiates IgE-dependent histamine release from human lung mast cells by an A2-purinoceptor mediated mechanism. *Biochem. Pharmacol.,* **33,** 3847–3852.

HUGHES, P.J., HOLGATE, S.T., ROATH, S. & CHURCH, M.K. (1983). The relationship between cyclic AMP changes and histamine release from basophil-rich human leucocytes. *Biochem. Pharmacol.,* **32,** 2557–2563.

INBE, H., WATANABE, S., MIYAWAKI, M., TANABE, E. & ENCINAS, J.A. (2004). Identification and characterization of a cell-surface receptor, P2Y15, for AMP and adenosine. *J. Biol. Chem.,* **279,** 19790–19799.

JOOS, G.F., O'CONNOR, B., ANDERSON, S.D., CHUNG, F., COCKCROFT, D.W., DAHLEN, B., DIMARIA, G., FORESI, A., HARGREAVE, F.E., HOLGATE, S.T., INMAN, M., LOTVALL, J., MAGNUSSEN, H., POLOSA, R., POSTMA, D.S., RIEDLER, J. & ERS TASK FORCE (2003). Indirect airway challenges. *Eur. Respir. J.,* **21,** 1050–1068.

KIM, S.A., MARSHALL, M.A., MELMAN, N., KIM, H.S., MULLER, C.E., LINDEN, J. & JACOBSON, K.A. (2002). Structure–activity relationships at human and rat A2B adenosine receptors of xanthine derivatives substituted at the 1-, 3-, 7-, and 8-positions. *J. Med. Chem.,* **45,** 2131–2138.

KONNARIS, K. & LLOYD, H.G (1996). Temple DM release of adenosine from human sensitised lung fragments and effect on antigen-induced mediator release. *Pulmon. Pharmacol.,* **9,** 141–148.

LEE, D.K., GRAY, R.D. & LIPWORTH, B.J. (2003). Adenosine monophosphate bronchial provocation and the actions of asthma therapy. *Clin. Exp. Allergy,* **33,** 287–294.

LONG, F., WANG, Y.X., LIU, L., CUI, R.Y. & JIANG, C.L. (2005). Rapid nongenomic inhibitory effects of glucocorticoids on phagocytosis and superoxide anion production by macrophages. *Steroids,* **70,** 55–61.

LUIJK, B., CASS, L. & LAMMERS, J.-W.J. (2003). The adenosine A2A-receptor is not involved in adenosine induced bronchoconstriction in asthmatics. *Eur. Resp. J.,* **22** (Suppl 45), 103s.

LUNELL, E., SVEDMYR, N., ANDERSSON, K.E. & PERSSON, C.G. (1983). A novel bronchodilator xanthine apparently without adenosine receptor antagonism and tremorogenic effect. *Eur. J. Respir. Dis.,* **64,** 333–339.

MANN, J.S., CUSHLEY, M.J. & HOLGATE, S.T. (1985). Adenosine-induced bronchoconstriction in asthma. Role of parasympathetic stimulation and adrenergic inhibition. *Am. Rev. Respir. Dis.,* **132,** 1–6.

MANN, J.S. & HOLGATE, S.T. (1985). Specific antagonism of adenosine-induced bronchoconstriction in asthma by oral theophylline. *Br. J Clin. Pharmacol.,* **19,** 685–692.

MANN, J.S., HOLGATE, S.T., RENWICK, A.G. & CUSHLEY, M.J. (1986a). Airway effects of purine nucleosides and nucleotides and release with bronchial provocation in asthma. *J. Appl. Physiol.,* **61,** 1667–1676.

MANN, J.S., HOLGATE, S.T., RENWICK, A.G. & CUSHLEY, M.J. (1986b). Airway effects of purine nucleosides and nucleotides and release with bronchial provocation in asthma. *J. Appl. Physiol.,* **61,** 1667–1676.

MANN, J.S., RENWICK, A.G. & HOLGATE, S.T. (1986c). Release of adenosine and its metabolites from activated human leucocytes. *Clin. Sci. (London),* **70,** 461–468.

MATHARU, A.L., MUNDELL, S.J., BENOVIC, J.L. & KELLY, E. (2001). Rapid agonist-induced desensitization and internalization of the A(2B) adenosine receptor is mediated by a serine residue close to the COOH terminus. *J. Biol. Chem.,* **276,** 30199–30207.

MUNDELL, S.J., MATHARU, A.L., KELLY, E. & BENOVIC, J.L. (2000). Arrestin isoforms dictate differential kinetics of A2B adenosine receptor trafficking. *Biochemistry,* **39,** 12828–12836.

NYCE, J.W. & METZGER, W.J. (1997). DNA antisense therapy for asthma in an animal model. *Nature,* **385,** 721–725.

OHTA, A. & SITKOVSKY, M. (2001). Role of G-protein-coupled adenosine receptors in downregulation of inflammation and protection from tissue damage. *Nature,* **414,** 916–920.

OKAYAMA, Y. & CHURCH, M.K. (1992). Comparison of the modulatory effect of ketotifen, sodium cromoglycate, procaterol and salbutamol in human skin, lung and tonsil mast cells. *Int. Arch. Allergy Immunol.,* **97,** 216–225.

PAUWELS, R.A. & VAN DER STRAETEN, M.E. (1987). An animal model for adenosine-induced bronchoconstriction. *Am. Rev. Respir. Dis.,* **136,** 374–378.

PERSIANI, S., D'AMATO, M., MAKOVEC, F., ARSHAD, S.H., HOLGATE, S.T. & ROVATI, L.C. (2001). Pharmacokinetics of andolast after administration of single escalating doses by inhalation in mild asthmatic patients. *Biopharm. Drug Dispos.,* **22,** 73–81.

PERSSON, C.G., ANDERSSON, K.E. & KJELLIN, G. (1986). Effects of enprofylline and theophylline may show the role of adenosine. *Life Sci.,* **38,** 1057–1072.

PETERS, S.P., SCHULMAN, E.S., SCHLEIMER, R.P., MACGLASHAN JR, D.W., NEWBALL, H.H. & LICHTENSTEIN, L.M. (1982). Dispersed human lung mast cells. Pharmacologic aspects and comparison with human lung tissue fragments. *Am. Rev. Respir. Dis.,* **126,** 1034–1039.

PHILLIPS, G.D., BAGGA, P.K., DJUKANOVIC, R. & HOLGATE, S.T. (1989a). The influence of refractoriness to adenosine 5′-monophosphate on allergen-provoked bronchoconstriction in asthma. *Am. Rev. Respir. Dis.,* **140,** 321–326.

PHILLIPS, G.D., FINNERTY, J.P. & HOLGATE, S.T. (1990a). 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, G.D. & HOLGATE, S.T. (1988a). Absence of a late-phase response or increase in histamine responsiveness after bronchial provocation with adenosine 5′-monophosphate in atopic and non-atopic asthma. *Clin. Sci. (London),* **75,** 429–436.

PHILLIPS, G.D. & HOLGATE, S.T. (1988b). Absence of a late-phase response or increase in histamine responsiveness after bronchial provocation with adenosine 5′-monophosphate in atopic and non-atopic asthma. *Clin. Sci. (London),* **75,** 429–436.

PHILLIPS, G.D. & HOLGATE, S.T. (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, G.D., NG, W.H., CHURCH, M.K. & HOLGATE, S.T. (1990). The response of plasma histamine to bronchoprovocation with methacholine, adenosine 5′-monophosphate, and allergen in atopic nonasthmatic subjects. *Am. Rev. Respir. Dis.,* **141,** 9–13.

PHILLIPS, G.D., RAFFERTY, P., BEASLEY, R. & HOLGATE, S.T. (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, G.D., SCOTT, V.L., RICHARDS, R. & HOLGATE, S.T. (1989b). Effect of nedocromil sodium and sodium cromoglycate against bronchoconstriction induced by inhaled adenosine 5′-monophosphate. *Eur. Respir. J.,* **2,** 210–217.

POLOSA, R. & HOLGATE, S.T. (1990). Comparative airway response to inhaled bradykinin, kallidin, and [des-Arg9]bradykinin in normal and asthmatic subjects. *Am. Rev. Respir. Dis.,* **142,** 1367–1371.

POLOSA, R., MAGRI, S., VANCHERI, C., ARMATO, F., SANTONOCITO, G., MISTRETTA, A. & CRIMI, N. (1997). Time course of changes in adenosine 5′-monophosphate airway responsiveness with inhaled heparin in allergic asthma. *J. Allergy Clin. Immunol.,* **99,** 338–344.

POLOSA, R., NG, W.H., CRIMI, N., VANCHERI, C., HOLGATE, S.T., CHURCH, M.K. & MISTRETTA, A. (1995). Release of mast-cell-derived mediators after endobronchial adenosine challenge in asthma. *Am. J. Respir. Crit. Care Med.,* **151,** 624–629.

POLOSA, R., PHILLIPS, G.D., RAJAKULASINGAM, K. & HOLGATE, S.T. (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, M.K. & HOLGATE, S.T. (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, M.K. & HOLGATE, S.T. (1993a). 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., RAJAKULASINGAM, K., PROSPERINI, G., MILAZZO, L.V., SANTONOCITO, G. & HOLGATE, S.T. (1993b). Cross-tachyphylactic airway response to inhaled bradykinin, kallidin and [desArg9]-bradykinin in asthmatic subjects. *Eur. Respir. J.,* **6,** 687–693.

POLOSA, R., RORKE, S. & HOLGATE, S.T. (2002). Evolving concepts on the value of adenosine hyperresponsiveness in asthma and chronic obstructive pulmonary disease. *Thorax,* **57,** 649–654.

PRIETO, L., BRUNO, L., GUTIERREZ, V., UIXERA, S., PEREZ-FRANCES, C., LANUZA, A. & FERRER, A. (2003). Airway responsiveness to adenosine 5′-monophosphate and exhaled nitric oxide measurements: predictive value as markers for reducing the dose of inhaled corticosteroids in asthmatic subjects. *Chest,* **124,** 1325–1333.

RAFFERTY, P., BEASLEY, R. & HOLGATE, S.T. (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.

RAJAKULASINGAM, K., CHURCH, M.K., HOWARTH, P.H. & HOLGATE, S.T. (1993). Factors determining bradykinin bronchial responsiveness and refractoriness in asthma. *J. Allergy Clin. Immunol.,* **92,** 140–142.

RAJAKULASINGAM, K., POLOSA, R., CHURCH, M.K., HOWARTH, P.H. & HOLGATE, S.T. (1994). Effect of inhaled frusemide on responses of airways to bradykinin and adenosine 5′-monophosphate in asthma. *Thorax,* **49,** 485–491.

REISSMANN, S., PINEDA, F., VIETINGHOFF, G., WERNER, H., GERA, L., STEWART, J.M. & PAEGELOW, I. (2000). Structure activity relationships for bradykinin antagonists on the inhibition of cytokine release and the release of histamine. *Peptides,* **21,** 527–533.

RICHARDS, R., PHILLIPS, G.D. & HOLGATE, S.T. (1989). Nedocromil sodium is more potent than sodium cromoglycate against AMP-induced bronchoconstriction in atopic asthmatic subjects. *Clin. Exp. Allergy,* **19,** 285–291.

RICHARDS, R., SIMPSON, S.F., RENWICK, A.G. & HOLGATE, S.T. (1988). Inhalation rate of sodium cromoglycate determines plasma pharmacokinetics and protection against AMP-induced bronchoconstriction in asthma. *Eur. Respir. J.,* **1,** 896–901.

ROACH, K.E., ALLY, D., FINNERTY, B., WATKINS, D., LITWIN, B.A., JANZ-HOOVER, B., WATSON, T. & CURTIS, K.A. (1998). The relationship between duration of physical therapy services in the acute care setting and change in functional status in patients with lower-extremity orthopedic problems. *Phys. Ther.,* **78,** 19–24.

RORKE, S. & HOLGATE, S.T. (2002). Targeting adenosine receptors: novel therapeutic targets in asthma and chronic obstructive pulmonary disease. *Am. J Respir. Med.,* **1,** 99–105.

RORKE, S., JENNISON, S., JEFFS, J.A., SAMPSON, A.P., ARSHAD, H. & HOLGATE, S.T. (2002). Role of cysteinyl leukotrienes in adenosine 5′-monophosphate induced bronchoconstriction in asthma. *Thorax,* **57,** 323–327.

RYZHOV, S., GOLDSTEIN, A.E., MATAFONOV, A., ZENG, D., BIAGGIONI, I & FEOKISTOV, 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.

SITARAMAN, S.V., SI-TAHAR, M., MERLIN, D., STROHMEIER, G.R. & MADARA, J.L. (2000). Polarity of A2b adenosine receptor expression determines characteristics of receptor desensitization. *Am. J. Physiol. Cell. Physiol.,* **278,** C1230–C1236.

SITARAMAN, S.V., WANG, L., WONG, M., BRUEWER, M., HOBERT, M., YUN, C.H., MERLIN, D. & MADARA, J.L. (2002). The adenosine 2b receptor is recruited to the plasma membrane and associates with E3KARP and Ezrin upon agonist stimulation. *J. Biol. Chem.,* **277,** 33188–33195.

SPICUZZA, L., BONFIGLIO, C. & POLOSA, R. (2003). Research applications and implications of adenosine in diseased airways. *Trends Pharmcol. Sci.,* **24,** 409–413.

SUMMERS, Q.A., HONEYWELL, R., RENWICK, A.G. & HOLGATE, S.T. (1990). The protective efficacy of inhaled, oral and intravenous nedocromil sodium against adenosine-5′-monophosphate-induced bronchoconstriction in asthmatic volunteers. *Pulmon. Pharmacol.,* **3,** 190–197.

SUN, C.X., YOUNG, H.W., MOLINA, J.G., VOLMES, J.B., SCHNERMANN, J. & BLACKBURN, M.R. (2005). A protective role for the A1 adenosine receptor in adenosine dependent pulmonary injury. *J. Clin. Invest.,* **115,** 35–43.

SYLVIN, H., VAN DER PLOEG, I. & ALVING, K. (2001). The effect of a bradykinin B2 receptor antagonist, NPC-567, on allergen-induced airway responses in a porcine model. *Inflamm. Res.,* **50,** 453–459.

THORNE, J.R., DANAHAY, H. & BROADLEY, K.J. (1996). Analysis of the bronchoconstriction responses to adenosine receptor agonists in sensitised guinea pig lungs and trachea. *Eur. J. Pharmacol.,* **316,** 263–271.

VAN DEN BERGE, M., KERSTJENS, H.A., MEIJER, R.J., DE REUS, D.M., KOETER, G.H., KAUFFMAN, H. & POSTMA, D.S. (2001). Corticosteroid induced improvement in the PC_{20} of adenosine monophosphate is more closely associated with reduction in airway inflammation than imporevement in the PC20 methcholine. *Am. J. Respir. Crit. Care Med.,* **164,** 1127–1132.

VAN DEN BERGE, M., KERSTJENS, H., DE REUS, D., KOETER, G., KAUFFMAN, H. & POSTMA, D. (2004). Provocation with adenosine 5′-monophosphate, but not methacholine, induces sputum eosinophilia. *Clin. Exp. Allergy,* **34,** 71–76.

WILSON, A.M., SIMS, E.J., ORR, L.C., ROBB, F. & LIPWORTH, B.J. (2003). An evaluation of short-term corticosteroid response in perennial allergic rhinitis using histamine and adenosine monophosphate nasal challenge. *Br. J Clin. Pharmacol.,* **55,** 354–359.

WOLBER, C. & FOZARD, J.R. (2005). The receptor mechanism mediating the contractile response to adenosine on lung parenchymal strips from actively sensitized allergen-challenged Brown Norway rats. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* (ePub ahead of print January 27).

ZENG, D., PROSPERINI, G., RUNO, C., SPICUZZA, L., CACCIOLA, R.R., DI MARIA, G. & POLOSA, R. (2004). Heparin attenuates symptoms and mast cell degranulation induced by AMP nasal provocation. *J. Allergy Clin. Immunol.,* **114,** 316–319.

ZHONG, H., BELADLINELLI, L., MAA, T., FEOKISTOV, I., BIAGGIONI, I. & ZENG, D. (2004a). 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. (2004b). Synergy between A_{2B} adenosine receptors and hypoxia in activating human lung fibroblasts. *Am. J. Respir. Cell. Mol. Biol.,* **32,** 2–8.

(Received January 25, 2005
Revised April 12, 2005
Accepted April 18, 2005
Published online 27 June 2005)