

REVIEW

Species differences in adenosine receptor-mediated bronchoconstrictor responses

J. R. FOZARD* and J. P. HANNON

Research Department, Novartis Pharma AG, CH 4002 Basel, Switzerland

Introduction

Airways hyperresponsiveness is a defining feature of asthma [1]. In the context of this key aspect of asthma aetiology, a particularly remarkable phenomenon is the sensitivity of the asthmatic airways to adenosine; essentially the airways of asthmatics respond with pronounced bronchoconstriction to both inhaled or intravenously administered adenosine, whereas the airways of nonasthmatics do not [2,3]. Although the underlying mechanism of the bronchoconstrictor response to adenosine is reasonably well understood, the reason for the striking up-regulation of the response in asthmatic airways and the receptor site(s) involved remains to be clarified [4–9]. To study the phenomenon in further detail it would clearly be an advantage to have an animal model of the airway response to adenosine in asthma. Bronchoconstrictor responses to adenosine have been studied in guinea-pigs, rabbits and rats. In this review, the key features of the bronchoconstrictor responses to adenosine in these three species are described and compared with those of the airway response to adenosine in asthmatics.

The bronchoconstrictor response to adenosine in asthmatics

Inhalation of aerosolized adenosine (or more usually adenosine 5'-monophosphate [AMP]—used for convenience since it has much greater water solubility and is broken down rapidly by 5'-nucleotidase to adenosine *in vivo*) is without effect on the airways of nonallergic, nonasthmatic subjects. When inhaled by atopic asthmatic subjects, adenosine (or AMP) causes a marked, dose-related bronchoconstriction, which develops within minutes, and lasts generally up to 1 h. There is no late response equivalent to that seen with allergen [2,10]. Tachyphylaxis occurs readily to AMP and is an example of homologous desensitization since it does not extend to histamine [11].

A variety of evidence points to the bronchoconstrictor response to adenosine being indirect, a consequence of activation of mast cells resident in the airways. Thus,

AMP-induced bronchoconstriction is accompanied by increased levels of histamine, PGD₂, tryptase (a highly specific marker for mast cells), but not β -glucuronidase (an indicator of macrophage stimulation), in the BAL fluid of mild atopic asthmatics; no such changes were seen in nonasthmatics [12]. Venous plasma histamine levels are also increased after AMP challenge [13]. Pharmacological analysis reveals that the bronchoconstrictor response to adenosine is inhibited ($\geq 80\%$) by pretreatment with the selective histamine H₁ receptor antagonists, terfenadine and astemizole [14–16]. The selective cyclooxygenase inhibitors, indomethacin and flurbiprofen, also partly inhibit AMP-induced bronchoconstriction [17,18] indicating that products of arachidonic acid metabolism also play a role, albeit minor, in adenosine-induced bronchoconstriction in asthmatic patients. Importantly, both adenosine- and AMP-induced bronchoconstriction can be prevented by pretreatment with disodium cromoglycate or nedocromil sodium [19,20], compounds which have in common the property of inhibiting mast cell degranulation and mediator release [21].

The *in vivo* evidence for the involvement of mast cell mediator release in adenosine-induced bronchoconstriction is supported by *in vitro* findings. Thus, bronchi from asthmatic patients showed an increased responsiveness to adenosine in comparison to those from nonasthmatic patients. The response on the bronchi from asthmatics could be blocked by a combination of leukotriene and histamine H₁ receptor antagonism [22].

The response of the asthmatic airways to adenosine appears to reflect activation of an extracellular purinoceptor(s). Thus, administration of dipyridamole, a compound which increases extracellular adenosine concentrations by inhibiting its facilitated reuptake through the cell membrane [23] enhances the effects of inhaled adenosine in asthmatic patients [24]. Further, adenosine-induced bronchoconstriction can be selectively (relative to histamine) inhibited by both inhaled [25] or orally [26] administered theophylline at doses generating plasma concentrations that are insufficient to inhibit phosphodiesterases but well above those needed to block A₁, A_{2A} and A_{2B} adenosine receptors [27]. Consistent with this, the responses of human bronchi from asthmatics to adenosine can be blocked by theophylline

Correspondence: J. R. Fozard, Research Department, Novartis Pharma AG, WSJ.386.508, CH-4002 Basel, Switzerland.

and the noncell-penetrating xanthine antagonist, 8-*p*-sulphophenyltheophylline (8-SPT) [22]. Finally, human lung mast cells can be activated by a variety of non cell-penetrating adenosine receptor agonists *in vitro* [28,29].

Evidence is accumulating that the receptor present on airway mast cells that mediates adenosine-induced bronchoconstriction in man is the A_{2B} receptor. Early *in vitro* studies implicated atypical 'A₂-like' receptors in adenosine-induced enhancement of mediator release from human lung mast cells [28,29]; with hindsight, the order of potency of the agonists used and the susceptibility to blockade by xanthine-based antagonists rule out the involvement of A₁, A_{2A} and A₃ receptors and are entirely consistent with mediation by the A_{2B} receptor. More recently, the human mast cell line, HMC-1 (which, although derived from a patient with mast cell leukaemia, has a neutral protease content similar to that of human lung mast cells [30]) has been found to express the A_{2A} and A_{2B} receptors; only stimulation of the latter resulted in cell activation and an increase in IL-8 secretion [31]. Studies using reverse transcriptase-polymerase chain reaction (RT PCR) confirmed the presence of A_{2A} and A_{2B} receptors in human mast cells [32]. In contrast, the presence of A₃ receptors could not be demonstrated in human lung mast cells either by *in situ* hybridization or by RT PCR [32]. The presence of A_{2B} receptors has also been demonstrated by immunofluorescence staining in the HMC-1 cell line and human lung mast cells isolated from the BAL fluid [33]. The A₁ receptor is reported not to be expressed in mast cells [7]. From the foregoing, neither the A₁, A_{2A} nor the A₃ receptor is likely to be involved in adenosine-induced bronchoconstriction. In view of this, the selective blockade by theophylline of adenosine-induced bronchoconstriction [25,26] can be used to support a role for the A_{2B} receptor since it is the only relevant purinoceptor which would be blocked at therapeutic blood concentrations of the drug [27].

On the basis of the above, five key criteria can be defined to represent the principal characteristics of the bronchoconstrictor response to adenosine in man:

- Upregulation by the disease process;
- Mast cell dependency;
- Selective blockade by theophylline;
- Involvement of A_{2B} receptors;
- Homologous desensitization (tachyphylaxis).

The characteristics of the bronchoconstrictor responses to adenosine in guinea-pigs, rabbits and rats will now be discussed and the extent to which they satisfy these key criteria assessed.

The bronchoconstrictor response to adenosine in the guinea-pig

Adenosine does not cause bronchoconstriction in normal conscious [34] or anaesthetized [35] guinea-pigs. However,

in conscious animals sensitized to ovalbumin, a bronchoconstrictor response to inhaled adenosine is observed [34]. The response was neither enhanced nor inhibited 24 h following ovalbumin challenge [36]. The mechanism underlying the bronchoconstrictor effect *in vivo* has not been evaluated.

The difference between nonsensitized and sensitized animals in terms of their airway reactivity to adenosine was confirmed by Broadley and colleagues in *in vitro* studies using perfused lung and tracheal smooth muscle preparations [37–39]. However, it bears emphasis that responses to adenosine were biphasic with bronchorelaxation being generally dominant at higher doses. The constrictor response to a second exposure to adenosine was consistently reduced (lungs) or converted to a relaxation (trachea) indicating tachyphylaxis [37]. Pharmacological analysis of the constrictor component of the response revealed it to be mimicked by 5'-N-ethylcarboxamidoadenosine (NECA), 2-chloroadenosine, R-N⁶-phenylisopropyladenosine (R-PIA), and N⁶-2-(4-aminophenyl)-ethyladenosine (APNEA) and to be resistant to blockade by 8-phenyltheophylline and 8-cyclopentyltheophylline; on this basis it was concluded that the response was mediated by the A₃ receptor [34,38,39]. The cell type(s) implicated has (have) not been identified. Histamine is not involved [38], which speaks against mast cell mediation of the constrictor component of the response. However, bronchoconstriction can be abolished by indomethacin [38] which implicates products of arachidonic acid metabolism in the response and would be consistent with the involvement of inflammatory cells.

In contrast to the results from the Broadley group, Kroell and colleagues were able to demonstrate adenosine-induced bronchoconstriction in the nonsensitized guinea-pig perfused lung [40]. Responses were neither attenuated by the histamine H₁ receptor antagonist, mepyramine, nor by disodium cromoglycate [40], but were blocked by theophylline [41] although only at concentrations (75–150 µM) at which nonselective adenosine receptor antagonism would be expected [27]. Contractile responses to adenosine and its agonists have also been reported on tracheas taken from nonsensitized animals [42–44]. Pharmacological analysis indicated mediation by the A₁ receptor and, as in the perfused lung, a major role for the products of arachidonic acid metabolism by cyclooxygenase but no involvement of histamine.

Although up-regulation of the bronchoconstrictor response to adenosine in sensitized guinea-pigs and the susceptibility to tachyphylaxis bear similarity to the situation in man; there are also important differences. Thus, biphasic responses of the airways to adenosine and, in particular, A₃ and/or A₁ receptor mediation of the bronchoconstrictor component represent fundamental differences

from the bronchoconstrictor response to adenosine in asthmatics (Table 1).

The bronchoconstrictor response to adenosine in the rabbit

Normal rabbits do not show a bronchoconstrictor response to even high doses of adenosine when given by inhalation [45]. In contrast, in an allergic rabbit model having many similarities with allergic asthma (rabbits immunized at birth with antigen [ragweed, house dust mite or *Alternaria tenuis*]) airway hyperresponsiveness develops to adenosine [45]. Bronchoconstriction is a consequence of activation of A₁ receptors present on the airway smooth muscle cells [45–48], which are up regulated as a consequence of the immunization procedure [47]. Responses can be blocked by theophylline [45] as would be expected from their being A₁ receptor mediated. In an elegant extension to these studies, Nyce and Metzger [49] confirmed that the A₁ receptor present in airway smooth muscle mediates adenosine-induced bronchoconstriction in the sensitized rabbit by treating animals with an antisense oligodeoxynucleotide targeted against the A₁ receptor. Animals selectively depleted of their airway smooth muscle A₁ receptors manifested a *pro rata* reduction of the bronchoconstrictor response to adenosine [49].

Up-regulation of the bronchoconstrictor response to adenosine in rabbits immunized from birth with antigen reflects closely the clinical situation where adenosine causes prominent bronchoconstriction in allergic asthmatics whereas nonasthmatics fail to respond. Unfortunately, the similarities end there. Thus, unlike in the rabbit, there is no evidence that the airway response to adenosine in humans involves the A₁ receptor; indeed, as discussed above, there is evidence from several sources to the contrary. Moreover, unlike in humans, bronchoconstriction in the sensitized rabbit involves a direct action on the smooth muscle of the airways and is independent of mast cell activation. The rabbit therefore cannot be considered a suitable species in

which to model the bronchoconstrictor responses to adenosine in the asthmatic airway (Table 1).

The bronchoconstrictor response to adenosine in the rat

The response of the rat airway to adenosine *in vivo* is markedly strain-dependent. In a series of studies carried out by Pauwels and colleagues, rats of the Brown Norway and Fischer 344 strains were poorly responsive to several adenosine receptor agonists, including adenosine [50,51]. In contrast, rats of the BDE strain responded to intravenous administration of adenosine receptor agonists with reproducible, though rather small bronchoconstrictor responses. The order of potency to induce bronchoconstriction was NECA > 2-chloroadenosine = R-PIA = adenosine > S-PIA [50]. NECA was used in the subsequent analysis of the mechanisms underlying the bronchoconstrictor effects of adenosine receptor activation in this model. Responses to NECA were inhibited by high doses of the muscarine receptor antagonist, atropine, the 5-HT₂ receptor antagonist, methysergide, the mast cell stabilizing agents, disodium cromoglycate and nedocromil sodium, and the histamine H₁ receptor antagonist, ketotifen. In contrast, responses were unaffected by theophylline at doses yielding concentrations within the therapeutic range. The authors concluded that the bronchoconstrictor effect of adenosine was mediated through an 'A₂-type' receptor and largely indirect involving both mast cells and postsynaptic vagal nerve endings [50].

In a subsequent report from the Pauwels group [52], the investigation of a new set of agonists delivered an order of potency for bronchoconstrictor potency in BDE rats of NECA = N⁶-cyclopentyladenosine (CPA) > APNEA > N⁶-cyclohexyladenosine (CHA) > R-PIA > 2-[p-(2-carboxyethyl) phenylamino]-5'-N-ethylcarboxamidoadenosine (CGS 21680). Bronchoconstrictor responses to NECA were inhibited by two nominally selective A₁ receptor antagonists KF15372 and KW3902. The conclusion drawn on the basis of these data was that adenosine-induced bronchoconstriction in the rat is most likely due to binding of adenosine to different

Table 1. Comparison of the extent to which the bronchoconstrictor responses to adenosine in guinea-pigs, rabbits and Brown Norway rats satisfy the key criteria for a model of adenosine-induced bronchoconstriction in asthma

Criterion	Guinea-pig	Rabbit	Brown Norway rat
Upregulation by the disease process	yes*	yes*	yes†
Mast cell dependency	no‡	no	yes
Selective blockade by theophylline	n.t	no§	yes
Involvement of adenosine A _{2B} receptors	no‡	no	yes
Homologous desensitization (tachyphylaxis)	yes‡	n.t	yes

n.t: not tested; *by active sensitization to allergen; †by mild allergic pulmonary inflammation; ‡based on *in vitro* data; §blockade but no evidence of selectivity.

receptor subtypes including the A₁, A_{2B} and A₃ subtypes [52].

Bronchoconstrictor responses to intravenous administration of the A₁/A₃ receptor agonist, APNEA, in BDE rats have also been described by Meade and colleagues [53]. The response was associated with the release of histamine and 5-HT into plasma leading to the conclusion that an A₃ receptor on mast cells was involved in the response. Mast cell degranulation, leading to increased levels of histamine in plasma has also been observed following A₃ receptor activation in both Wistar and Sprague Dawley rats [54–56]. It should be noted, however, that this was not associated with bronchoconstriction in rats of the Sprague Dawley strain [54]. On the basis of the effects of the highly selective A₁ receptor agonist, 2-chloro-N⁶-cyclopentyladenosine (CCPA), Meade *et al.* [53] also suggested, that an A₁ receptor-mediated component was capable of inducing bronchoconstriction in the BDE rat independently of mast cell A₃ receptor activation.

It cannot be over-emphasized that the above observations relate to the analysis of the responses to the adenosine analogues, NECA, APNEA and CCPA. The response to adenosine, even in the BDE rat, is minimal [50] and hence one might reasonably question the relevance of studies with these analogues to the human clinical situation where adenosine is used to initiate the bronchoconstrictor response.

A new approach to modelling the bronchoconstrictor response to adenosine in asthma using the rat has recently been described in presentations to the British Pharmacological Society [57–59]. The strategy was to explore the role of active sensitization and subsequent challenge with antigen in defining the response of the airways to adenosine. The Brown Norway rat was chosen for these studies since this strain can be readily and reproducibly sensitized to antigen with high specific IgE production and manifests early and late bronchoconstrictor responses to allergen challenge [60–62].

In confirmation of the findings of Pauwels and colleagues described above, adenosine was only a very weak bronchoconstrictor when administered intravenously to naive (i.e. nonsensitized) Brown Norway rats. Similarly, in rats actively sensitized to ovalbumin, adenosine proved to be only a weak bronchoconstrictor. However, 3 h following intratracheal challenge with ovalbumin (0.3 mg kg⁻¹), which *per se* did not induce bronchoconstriction, bronchoconstrictor responses to adenosine were markedly and selectively (*vis a vis* those to 5-hydroxytryptamine (5-HT)) increased (Fig. 1). Histological examination of the lungs, at this time point, revealed signs of inflammation quantified as the presence of increased perivascular polymorphonuclear leucocyte infiltration, with occasional perivascular oedema, and a significant increase in eosinophil

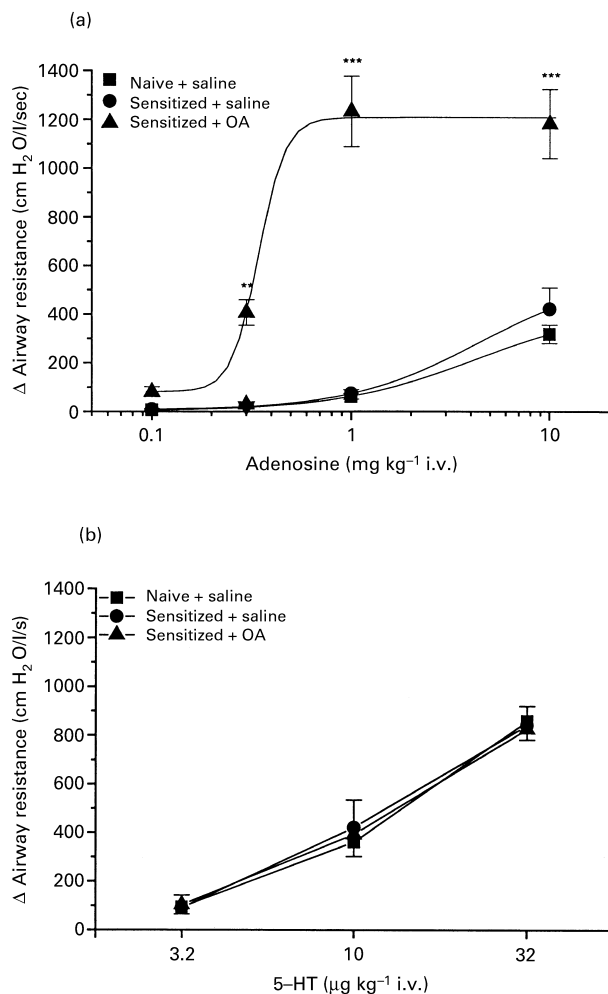


Fig. 1. Comparison of the bronchoconstrictor effect of intravenously administered adenosine (A) and 5-HT (B) in nonsensitized (naive) Brown Norway rats, or ovalbumin (OA) sensitized animals challenged with saline or OA (0.3 mg kg⁻¹) intratracheally, 3 h prior to establishing the dose-response curves. Results are expressed as means $\pm$ SEM ($n = 4-10$). *** $P < 0.001$ that the value is significantly different from the equivalent value in either the naive or saline-challenged groups. For further details see [57].

numbers in both tertiary bronchi and perivascular tissue [Hannon, unpublished data]. The degree of hyperresponsiveness to adenosine was a function both of the dose of ovalbumin and the time of pretreatment: for instance, 24 h after challenge with ovalbumin (0.3 mg kg⁻¹) the sensitivity of the airways to adenosine was close to control values despite a marked pulmonary inflammatory response [57].

The response to adenosine augmented 3 h following challenge with ovalbumin (0.3 mg kg⁻¹) has been analysed in detail [57–59]. The fact that bronchoconstrictor responses to NECA, a nonselective adenosine receptor

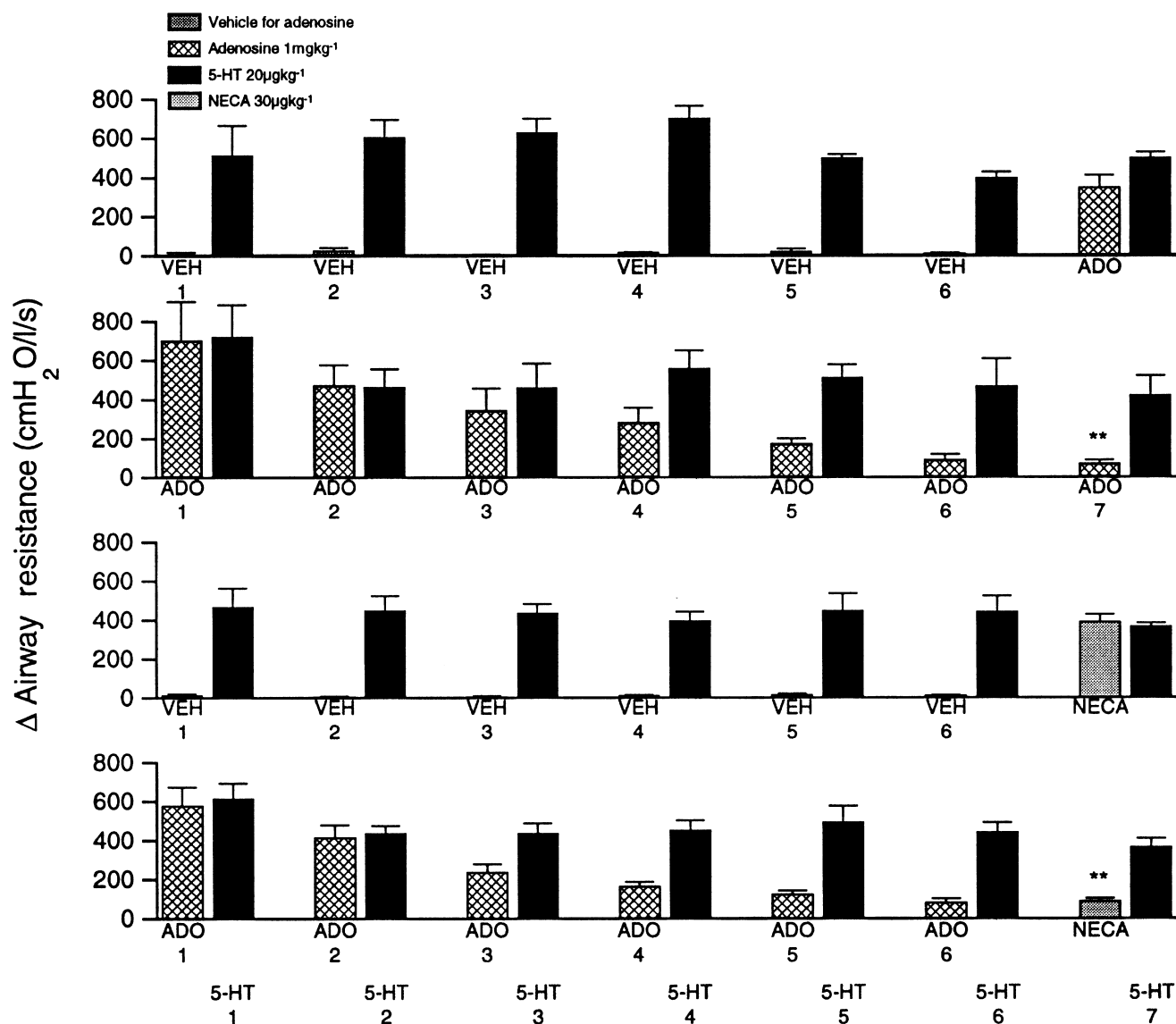


Fig. 2. Bronchoconstrictor responses to repeated (at 5 min intervals) intravenous injection of adenosine (ADO, 1mgkg^{-1}), the vehicle for adenosine (VEH) or 5-HT ($20\mu\text{gkg}^{-1}$) in Brown Norway rats actively sensitized to ovalbumin and challenged 3 h prior to starting the injection sequences with ovalbumin (0.3mgkg^{-1} , intratracheally). Evidence of cross tachyphylaxis with N-ethylcarboxamidoadenosine (NECA, $30\mu\text{gkg}^{-1}$ i.v.) is also demonstrated. Results are expressed as means $\pm$ SE mean ($n=4-5$). ** $P<0.01$ that the response to adenosine or NECA differs significantly from the equivalent control response.

agonist, were, like those to adenosine, augmented following ovalbumin challenge whereas those to selective A_1 , A_{2A} or A_3 receptor agonists were minimally affected, suggests involvement of the A_{2B} receptor in the response [58]. The conclusion is strengthened by the fact that bronchoconstrictor responses to adenosine could be suppressed selectively by both 8-SPT or theophylline, which block A_1 , A_{2A} and A_{2B} receptors, but not by antagonists selective for A_1 or A_{2A} receptors [58]. Tachyphylaxis readily occurs to adenosine;

moreover animals rendered tachyphylactic to adenosine were less responsive to NECA, whereas responses to 5-HT were maintained (Fig. 2).

Persuasive evidence of mast cell involvement comes from the demonstration of marked increases in plasma levels of both histamine and 5-HT following adenosine administration, and attenuation of these increases by pre-treatment with 8-SPT, disodium cromoglycate or wortmannin, a selective inhibitor of phosphatidylinositol-3 kinase, at

doses which inhibit the bronchoconstrictor responses to adenosine [57–59, Hannon, unpublished data]. The selective suppression of the bronchoconstrictor responses to adenosine by pretreatment with the 5-HT₂ receptor antagonist, methysergide [57], and in animals depleted of mast cells by subchronic treatment with compound 48/80 [Hannon, unpublished data] further support a key role for the mast cell in the responses to adenosine augmented following allergen challenge.

These data demonstrate a marked and selective up-regulation of the response to adenosine in actively sensitized Brown Norway rats following challenge with antigen. The response occurs against a background of mild allergic pulmonary inflammation, is a consequence of A_{2B} receptor-mediated activation, is mast cell dependent, exhibits tachyphylaxis and can be blocked selectively by theophylline. It thus satisfies all the key criteria defining a model of the response to adenosine in asthmatic patients (Table 1).

Conclusion

Of the three animal species in which the bronchoconstrictor response to adenosine has been evaluated in detail, the Brown Norway rat actively sensitized to ovalbumin and subjected to relatively mild antigen challenge provides the model which most closely resembles the response to adenosine in human asthma (Table 1). A further similarity is that in asthmatic patients, as in the Brown Norway rat, augmentation of the response to adenosine is particularly prominent 3 h after allergen challenge but is no longer evident at 24 h [63]. Taken together these observations testify to the impressive similarity of bronchoconstriction to adenosine in the rat model to the response of asthmatic airways to adenosine.

However, when considering the merits of any animal model one must also be conscious of its limitations. Thus, whilst bronchoconstriction in the Brown Norway rat model does accurately reflect many aspects of the response to adenosine in the atopic asthmatic patient, it is, nevertheless, the consequence of an acute and not chronic allergic pulmonary inflammatory response. Moreover, the model is, by definition, unlikely to mirror accurately the mechanism of the augmented adenosine-induced bronchoconstrictor responses seen in intrinsic asthmatics or nonatopic smokers [64,65]. Furthermore, the mediator of the bronchoconstrictor response to adenosine in the Brown Norway rat is 5-HT and not, as in the asthmatic patient, mainly histamine [14–16]. Finally, the relative distribution and pharmacological profile of adenosine receptor subtypes between rat and human remains largely undefined and may turn out to be a complicating factor in extrapolating data from the rat model to man.

Notwithstanding its potential deficiencies, the Brown Norway rat model provides a novel and simple way of profiling adenosine A_{2B} receptor antagonists *in vivo*. As such, it may prove useful in the profiling of adenosine A_{2B} receptor antagonists as novel treatments for asthma [27,30,31].

References

- 1 Woolcock AJ. Definitions and clinical classification. In: Barnes PJ, Grunstein MM, Leff AR, Woolcock AJ (eds). *Asthma*. Philadelphia: Lippencourt-Raven, 1997: 27–32.
- 2 Cushley MJ, Tattersfield AE, Holgate ST. Inhaled adenosine and guanosine on airway resistance in normal and asthmatic subjects. *Br J Clin Pharmacol* 1983; 5:161–5.
- 3 Drake I, Routledge PA, Richards R. Bronchospasm induced by intravenous adenosine. *Hum Exp Toxicol* 1994; 13:263–5.
- 4 Broadley KJ, Purines. In: Raeburn D, Gienbycz MA. eds. *Airways Smooth Muscle: Neurotransmitters, Amines, Lipid Mediators and Signal Transduction*. Basel: Birkhäuser, 1996; 271–307.
- 5 Phillips GD, Holgate ST. Adenosine and the airways in asthma. In: Busse WW, Hogate ST. eds. *Asthma and Rhinitis* Oxford: Blackwell Scientific, 1995: 851–7.
- 6 Jacobson MA, Bai TR. The role of adenosine in asthma. In: Jacobson KA, Jarvis MF eds. *Purinergic Approaches in Experimental Therapeutics* Chichester: Wiley-Liss, 1997: 315–31.
- 7 Marquardt DL. Adenosine. In: Barnes PJ, Grunstein MM, Leff AR, Woolcock AJ (eds). *Asthma*. Philadelphia: Lippencourt-Raven, 1997: 585–91.
- 8 Polosa R, Holgate ST. Adenosine bronchoprovocation: a promising marker of allergic inflammation in asthma? *Thorax* 1997; 52:919–23.
- 9 Forsythe P, Ennis M. Adenosine, mast cells and asthma. *Inflamm Res* 1999; 48:301–7.
- 10 Phillips GD, Holgate ST. 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* 1988; 75:429–36.
- 11 Daxun Z, Rafferty P, Richards R, Summerell S, Holgate ST. Airway refractoriness to adenosine 5'-monophosphate after repeated inhalation. *J Allergy Clin Immunol* 1989; 83:152–8.
- 12 Polosa R, Ng WH, Crimi N, Vancheri C, Holgate ST, Mistretta A. Release of mast-cell-derived mediators after endobronchial adenosine challenge in asthma. *Am J Respir Crit Care Med* 1995; 151:624–9.
- 13 Phillips DG, Ng WH, Church MK, Holgate ST. The response of plasma histamine to bronchoprovocation with methacholine, adenosine 5'-monophosphate, and allergen in atopic non-asthmatic subjects. *Am Rev Respir Dis* 1990; 141:9–13.
- 14 Phillips GD, Rafferty P, Beasley R, Holgate ST. The effect of oral terfenadine on the bronchoconstrictor response to inhaled histamine and adenosine 5'-monophosphate in non-atopic asthma. *Thorax* 1987; 42:939–45.
- 15 Rafferty P, Beasley R, Southgate P, Holgate ST. The role of histamine in allergen and adenosine-induced bronchoconstriction. *Int Arch Allergy Appl Immunol* 1987; 82:292–4.

- 16 Polosa R, Phillips GD, Rajakulasingam K, Holgate ST. 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* 1991; 87:939-47.
- 17 Phillips GD, Holgate ST. The effect of oral terfenadine alone and in combination with flurbiprofen on the bronchoconstrictor response to inhaled adenosine 5'-monophosphate in non-atopic asthma. *Am Rev Resp Dis* 1989; 139:463-9.
- 18 Crimi N, Palermo F, Polosa R, Oliveri R, Maccarrone C, Palermo B, Mistretta A. Effect of indomethacin on adenosine-induced bronchoconstriction. *J Allergy Clin Immunol* 1988; 83:921-5.
- 19 Phillips GD, Scott VL, Richards R, Holgate ST. Effect of nedocromil sodium and sodium cromoglycate against bronchoconstriction induced by inhaled adenosine 5'-monophosphate. *Eur Respir J* 1989; 2:210-7.
- 20 Richards R, Phillips GD, Holgate ST. Nedocromil sodium is more potent than sodium cromoglycate against AMP-induced bronchoconstriction in atopic asthmatic subjects. *Clin Exp Allergy* 1989; 19:285-91.
- 21 Eady RP, Norris AA. Nedocromil sodium and sodium cromoglycate: Pharmacology and putative modes of action. In: Kay AB. ed. *Allergy and Allergic Diseases* Oxford: Blackwell Scientific, 1997: 584-95.
- 22 Björck T, Gustafsson LE, Dahlen S. Isolated bronchi from asthmatics are hyperresponsive to adenosine, which apparently acts indirectly by liberation of leukotrienes and histamine. *Am Rev Respir Dis* 1992; 145:1087-91.
- 23 Turnheim K, Plank B, Kolassa N. Inhibition of adenosine uptake in human erythrocytes by adenosine-5'-carboxyamides, xylosyladenine, dipyridamole, hexobendine and p-nitrobenzylthioguanosine. *Biochem Pharmacol* 1978; 27:2191-7.
- 24 Cushley MJ, Tallant N, Holgate ST. The effect of dipyridamole on histamine- and adenosine-induced bronchoconstriction in normal and asthmatic subjects. *Eur J Respir Dis* 1985; 67:185-92.
- 25 Cushley MJ, Tattersfield AE, Holgate ST. Adenosine-induced bronchoconstriction in asthma. Antagonism by inhaled theophylline. *Am Rev Respir Dis* 1984; 129:380-4.
- 26 Mann JS, Holgate ST. Specific antagonism of adenosine-induced bronchoconstriction in asthma by oral theophylline. *Br J Clin Pharmacol* 1985; 19:685-92.
- 27 Fozard JR, Hannon JP. Adenosine receptor ligands: potential as therapeutic agents in asthma and COPD. *Pulm Pharmacol Ther* 1999; 12:111-4.
- 28 Peachell PT, Lichtenstein LM, Scheimer RP. Regulation of human basophil and lung mast cell function by adenosine. *J Pharmacol Exp Ther* 1991; 256:717-26.
- 29 Hughes PJ, Holgate ST, Church MK. Adenosine inhibits and potentiates IgE-dependent histamine release from human lung mast cells by an A₂-purinoceptor mediated mechanism. *Biochem Pharmacol* 1984; 33:3847-52.
- 30 Feoktistov I, Polosa R, Holgate ST, Biaggioni I. Adenosine A_{2B} receptors: a novel therapeutic target in asthma? *Trends Pharmacol Sci* 1998; 19:148-53.
- 31 Feoktistov I, Biaggioni I. Adenosine A_{2B} receptors evoke interleukin-8 secretion in human mast cells. An enprofylline-sensitive mechanism with implications for asthma. *J Clin Invest* 1995; 96:1979-86.
- 32 Walker BAM, Jacobson MA, Knight DA, et al. Adenosine A₃ receptor expression and function in eosinophils. *Am J Respir Cell Mol Biol* 1997; 16:531-7.
- 33 Feoktistov I, Sheller JR, Vallejo V, Biaggioni I. Immunological identification of A_{2b} receptors in human lung mast cells. *Drug Dev Res* 1996; 37:146.
- 34 Thorne JR, Broadley KJ. Adenosine-induced bronchoconstriction in conscious hyperresponsive and sensitised guinea-pigs. *Am J Respir Crit Care Med* 1994; 149:392-9.
- 35 Breschi MC, Nieri P, Lazzeri N, Macchia M. Potentiation by adenosine of histamine-induced bronchospasm in anaesthetized guinea-pig: Receptor subtype/s involved. *Drug Dev Res* 1998; 48:17-22.
- 36 Lewis CA, Broadley KJ. Airway hyper- or hyporeactivity to inhaled spasmogens 24h after ovalbumin challenge of sensitised guinea-pigs. *Br J Pharmacol* 1995; 116:2351-8.
- 37 Thorne JR, Broadley KJ. Adenosine-induced bronchoconstriction of isolated lung and trachea from sensitised guinea-pigs. *Br J Pharmacol* 1992; 106:978-85.
- 38 Thorne JR., Danahay, H. & Broadley, KJ. Analysis of the bronchoconstrictor responses to adenosine receptor agonists in sensitised guinea-pig lungs and trachea. *Eur J Pharmacol* 1996; 316:263-71.
- 39 Kehoe R. & Broadley, KJ. Bronchoconstriction by adenosine of sensitised guinea-pig trachea is mediated via A₃ receptors. *Pharmacol Commun*, 1996; 7:293-9.
- 40 Kroell F, Karlsson JA, Ryrfeldt A, Persson CGA. Adenosine-induced bronchoconstriction in the guinea pig isolated lung: Interaction with theophylline and enprofylline. *Pulm Pharmacol* 1988; 1:85-92.
- 41 Kroell F, Karlsson JA, Persson CGA, Ryrfeldt A. Interactions between xanthines, mepyramine and adenosine in the guinea pig lung. *Curr Clin Pract Ser* 1985; 19:193-6.
- 42 Ghai G, Zimmerman MB, Hopkins MF. Evidence for A₁ and A₂ receptors in guinea pig-trachea. *Life Sci* 1987; 41:1215-24.
- 43 Farmer SG, Canning BJ, Wikins DE. Adenosine receptor-mediated contraction and relaxation of guinea-pig isolated tracheal smooth muscle: Effects of adenosine antagonists. *Br J Pharmacol* 1988; 95:371-8.
- 44 Advenier C, Bidet D, Floch-Saint-Aubin A, Renier A. Contribution of prostaglandins and thromboxanes to the adenosine and ATP-induced contraction of guinea-pig isolated trachea. *Br J Pharmacol* 1982; 77:39-44.
- 45 Ali S, Mustafa SJ, Metzger WJ. Adenosine-receptor mediated bronchoconstriction and bronchial hyperresponsiveness in an allergic rabbit model. *Am J Physiol* 1994; 266:L271-L277.
- 46 El-Hashim A, D'agostino B, Matera MG, Page C. Characterisation of adenosine receptors involved in adenosine-induced bronchoconstriction in allergic rabbits. *Br J Pharmacol* 1996; 119:1262-8.
- 47 Ali S, Mustafa SJ, Metzger WJ. Adenosine-induced bronchoconstriction and contraction of airway smooth muscle from

- allergic rabbits with late phase airway obstruction: evidence for an inducible A₁ receptor. *J Pharm Exp Ther* 1994; 268:1328–34.
- 48 El-Hashim A, Banner KH, Paul W, Page C. Effects of dexamethasone on airway hyperresponsiveness to the adenosine A₁ receptor agonist cyclopentyladenosine in an allergic rabbit model. *Br J Pharmacol* 1999; 126:1513–21.
- 49 Nyce JW, Metzger WJ. DNA antisense therapy for asthma in an animal model. *Nature* 1997; 385:721–5.
- 50 Pauwels R, Van Der Straeten ME. An animal model of adenosine-induced bronchoconstriction. *Am Rev Resp Dis* 1987; 136:374–8.
- 51 Pauwels R, Joos GF, Kips JC, Peleman RA. Mechanisms of adenosine-induced bronchoconstriction: an animal model. *Drug Dev Res* 1993; 28:318–21.
- 52 Pauwels R, Joos G. Characterisation of the adenosine receptors in airways. *Arch Int Pharmacodyn* 1995; 329:151–60.
- 53 Meade C, Mierau J, Indepencia L, Ensinger HA. *In vivo* role of the adenosine A₃ receptor: N⁶-2-(4-aminophenyl) ethyladenosine induces bronchospasm in BDE rats by a neurally mediated mechanism involving cells resembling mast cells. *J Pharm Exp Therap* 1996; 297:1148–56.
- 54 Hannon JP, Pfannkuche H-J, Fozard JR. A role for mast cells in adenosine A₃ receptor-mediated hypotension in the anaesthetised rat. *Br J Pharmacol* 1995; 115:945–52.
- 55 Fozard JR, Pfannkuche H-J, Schuurmann H-J. Mast cell degranulation following adenosine A₃ receptor activation in rats. *Eur J Pharmacol* 1999; 6 (298):293–7.
- 56 Van Schaick EA, Jacobson KA, Kim HO, Ijzerman AP, Danhof M. Haemodynamic effects and histamine release elicited by the selective adenosine A₃ receptor agonist 2-Cl-IB-MECA in conscious rats. *Eur J Pharmacol* 1996; 308:311–4.
- 57 Hannon JP, Tigani B, Mazzoni L, Fozard JR. Mechanism of airway hyperreactivity to adenosine induced by allergen challenge in actively sensitised Brown Norway rats. *Br J Pharmacol* 1999; 127:76P.
- 58 Hannon JP, Tigani B, Mazzoni L, Fozard JR. The receptor mediating airway hyperreactivity to adenosine induced by allergen challenge in actively sensitised Brown Norway rats. *Br J Pharmacol* 1999; 127:77P.
- 59 Tigani B, Hannon JP, Mazzoni L, Fozard JR. Effects of wortmannin on antigen-induced airway inflammation and bronchoconstrictor responsiveness in actively sensitised Brown Norway rats. *Br J Pharmacol* 1999; 128:111P.
- 60 Pauwels R, Bazin H, Platteau B, Van Der Straeten M. The influence of antigen dose on IgE production in different rat strains. *Immunology* 1979; 36:151–7.
- 61 Elwood W, Lötval JO, Barnes PJ, Fan Chung K. Characterisation of allergen-induced bronchial hyperresponsiveness and airway inflammation in actively sensitised Brown Norway rats. *J Allergy Clin Immunol* 1991; 88:951–60.
- 62 Peers SH, Duncan GS, Flower RJ. Development of specific antibody and *in vivo* response to antigen in different rat strains: effect of dexamethasone and importance of endogenous corticosteroid. *Agents Actions* 1993; 39:174–81.
- 63 Aalbers R, Kauffman HF, Koeter GH, Postma DS, De Vries K, De Monchy JGR. Dissimilarity in methacholine and adenosine 5'-monophosphate responsiveness 3 and 24h after allergen challenge. *Am Rev Respir Dis* 1991; 144:352–7.
- 64 Pin I, Hepperle MJ, Wong BJO, Ramsdale EH, Hargreave FE. Methacholine and adenosine monophosphate (AMP) airway hyperresponsiveness in asthmatics and smokers with chronic airflow limitation. *Am Rev Respir Dis* 1991; 143:A413.
- 65 Oosterhoff Y, De Jong JW, Jansen AM, Koeter GH, Postma DS. Airway responsiveness to adenosine 5'-monophosphate in chronic obstructive pulmonary disease is determined by smoking. *Am Rev Resp Dis* 1993; 147:553–866.