Characterization of adenosine receptor(s) involved in adenosine-induced bronchoconstriction in an allergic mouse model

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Fan, Ming, Weixi Qin, and S. Jamal Mustafa. 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, 2003.—We recently reported that adenosine caused bronchoconstriction and enhanced airway inflammation in an allergic mouse model. In this study, we further report the characterization of the subtype of adenosine receptor(s) involved in bronchoconstriction, 5'-N(ethylcarboxamido)adenosine (NECA), a nonselective adenosine agonist, elicited bronchoconstriction in a dose-dependent manner. Little effects of N6-cyclopentyladenosine (A1-selective agonist) and 2-p-(2-carboxyethyl)phenethylamino-5'-N-ethylcarboxamidoadenosine (A2A-selective agonist) compared with NECA were observed in this model. 2-Chloro-N6-(3-iodobenzyl)-9-[5-(methylcarbamoyl)-β-d-ribofuranosyl]adenosine, an A3-selective receptor agonist, produced a dose-dependent bronchoconstrictor response, which was blocked by selective A3 antagonist 2,3-diethyl-4,5-dipropyl-6-phenylpyridine-3-thiocarbamoyl-5-carboxylate (MRS1523). However, MRS1523 only partially inhibited NECA-induced bronchoconstriction. Neither selective A1 nor A2A antagonists affected NECA-induced bronchoconstriction. Enprofylline, a relatively selective A2B receptor antagonist, blocked partly NECA-induced bronchoconstriction. Furthermore, a combination of enprofylline and MRS1523 completely abolished NECA-induced bronchoconstriction. Using RT-PCR, we found that all four adenosine receptor subtypes are expressed in control lungs. Allergen sensitization and challenge significantly increased transcript levels of the A2B and A3 receptors, whereas the A1 receptor message decreased. No change in transcript levels of A2A receptors was observed after allergen sensitization and challenge. These findings suggest that A2B and A3 adenosine receptors play an important role in adenosine-induced bronchoconstriction in our allergic mouse model. Finally, whether the airway effects of the receptor agonists/antagonists are direct or indirect needs further investigations.

Adenosine agonists; adenosine antagonists; mouse lung; asthma

Among the many actions of adenosine, several lines of evidence suggest a contribution of adenosine to the pathophysiology of asthma. Adenosine is present in high concentrations in the bronchoalveolar lavage fluid of asthmatics (8). Airway preparations from allergic asthmatic subjects are more sensitive to the contractile responses of adenosine and related analogs (4). Inhaled adenosine causes bronchoconstriction in asthmatics and allergic nonasthmatics compared with normal subjects (24).

The response of the airway tissues to adenosine involves the activation of extracellular adenosine receptor(s). Theophylline, a nonselective adenosine receptor antagonist, selectively (relative to histamine) inhibited adenosine-induced bronchoconstriction at doses generating plasma concentrations that are insufficient to inhibit phosphodiesterases but well above those needed to block adenosine receptors (22). Moreover, both rebound hyperresponsiveness to adenosine and exacerbation of symptoms have been reported when chronic theophylline therapy was withdrawn (36).

Adenosine receptors are seven-transmembrane-spanning receptors that are coupled to the effector system through heterotrimeric G proteins. Four subtypes of adenosine receptors, A1, A2A, A2B, and A3, have been identified based on the order of agonist potency and antagonist affinity, G protein coupling, cellular responses, and receptor cloning studies (11). Each of these receptor subtypes is expressed in lung and may have a role in asthma.

Most evidence indicates that activation of the A1 receptors, and to some extent the A3 receptors, induces adenylyl cyclase inhibition with subsequent decrease in adenosine 3',5'-cyclic monophosphate (cAMP) level. This intracellular signal induces contraction of the airway smooth muscle. In contrast, when adenosine activates the A2A receptors, cAMP level increases, which causes airway relaxation (29). Adenosine A2B receptors have long been known to be coupled to Gs protein, which mediates the activation of adenylyl cyclase (12, 33). Recent studies indicate that A2B receptors are also coupled to Gq and result in Ca2+ mobilization and mitogen-activated protein kinase activation (20, 21). Also, the A2 receptors have been suggested to mediate the facilitation of antigen-induced release of bronchoconstrictor mediators through inositol triphosphate and the Ca2+ pathway (34). Despite the pivotal role that adenosine receptors play in bronchoconstriction and inflammatory reactions under allergic conditions, the subtype(s) of adenosine receptors mediating

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bronchoconstriction remain poorly defined. The accumulated evidence shows that the receptor subtype varies widely among species, for example, the involvement of A3 receptors in the guinea pig (18), A2 receptors on airway smooth muscle cell in the rabbit (28), and a combination of A1, A2B, and A3 receptors in the BDE rat (31). In mice, our data (9) and those of others (6) have shown that exogenous and endogenous adenosine mediates bronchoconstriction and airway hyperresponsiveness through specific adenosine receptors, but the relative contribution of each adenosine receptor subtype mediating bronchoconstriction in mice is still unresolved.

The present study was initiated following our previous study demonstrating that ragweed sensitization and challenge of mice induced a profound increase in bronchoconstrictor response to adenosine and lung inflammation (9). Here, we investigated the involvement of the subtype of adenosine receptor(s) mediating bronchoconstriction to adenosine using selective adenosine receptor agonists and antagonists and studied a profile of adenosine receptor expression in allergic mouse lung using RT-PCR.

MATERIALS AND METHODS

**Mice and Sensitization**

Male BALB/c mice, 6–8 wk of age and free of specific pathogens, were obtained from Harlan Laboratories (Indianapolis, IN). The animals were maintained on a ragweed-free diet. All experimental animals used in this study were under a protocol approved by the Institutional Animal Care and Use Committee of East Carolina University.

Sensitization was performed according to a method described previously (9). In brief, mice were sensitized with two intraperitoneal injections of ragweed allergen (Greer Laboratories, Lenoir, NC), 200 μg per dose with 200 μl of Imject alum (Pierce Laboratories, Rockford, IL) on days 1 and 6. Nonsensitized control animals received only the Imject alum with the same volumes. After sensitization, the mice were placed in a Plexiglas chamber and challenged with 0.5% aerosolized ragweed or with 0.9% saline as a control, via an ultrasonic nebulizer (Devilbiss, Somerset, PA) for 20 min both in the morning and afternoon on days 11, 12, and 13.

**Experimental Protocol**

Airway responsiveness to adenosine agonists. Airway responsiveness was assessed with whole body plethysmograph (Max II; Buxco, Troy, NY). This system estimates total pulmonary air flow using a dimensionless parameter known as enhanced pause (Penh). Pressure differences were used to determine its effect on basal bronchial tone. Also, the effect of vehicle per se on Penh was examined.

RT-PCR. Mice were immunized with the protocol described above. Twenty-four hours after the last challenge with aerosolized allergen or 0.9% saline, mice were killed by injection (0.1 ml pentobarbitone sodium 200 mg/ml ip). Lungs were taken and immediately frozen in liquid nitrogen. Total RNA was isolated from the lungs of both ragweed-sensitized and -challenged mice and control animals with TRIzol reagent (Roche Diagnostics, Indianapolis, IN) according to the manufacturer’s instructions, and RT-PCR was conducted as described earlier (5). Briefly, for the RT reaction, 5 μg of purified total RNA were reverse transcribed in the presence of an anchored oligo-p (dT)15 primer, by use of avian myeloblastosis virus RT (Roche Diagnostics) based on the manufacturer’s recommendations. Sets of specific primers for adenosine receptors for A1 (37), A2A (15), A2B (5), and A3 (3) are presented in Table 1.

Single-stranded cDNA products were denatured and subjected to PCR amplification (35 cycles). Each PCR cycle consisted of denaturing at 94°C for 5 min (for A1 and A2B receptors) and at 94°C for 3 min (for A2A and A3 receptors), annealing at 60°C for 1 min for A2A, A3, and A2B receptors and at 65°C for 30 s (for A1 receptor), extension at 72°C for 2 min (for A1 and A2B receptors) and at 72°C for 1 min (for A2A and A3 receptors). A final extension of 72°C for 7 min (for A1 and A2B receptors) and of 72°C for 10 min (for A2A and A3 receptors) was added after the 35th amplification cycle.

The reaction mixture contained 0.2 mM deoxynucleotide 5’-triphosphate, 2.5 U/100 μl Taq DNA polymerase (Roche Diagnostics), and 1× PCR buffer (1.5 mM MgCl2) with GeneAmp PCR system (model 9600, Perkin-Elmer). Amplification of β-actin mRNA served as the control. The PCR products were separated on 2% agarose gels with ethidium bromide. The resulting data were analyzed using FluorChem digital Imaging System with AlphaEaseFC software (Alpha Innotech, San Leandro, CA). The relative expression level of adenosine receptor mRNA was calculated compared with β-actin. Results are expressed as means ± SE.
Table 1. PCR primers and PCR product sizes

<table>
<thead>
<tr>
<th>Primer</th>
<th>Nucleotides</th>
<th>Sequence</th>
<th>Product Sizes</th>
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<tr>
<td>A1 Forward</td>
<td>295–314</td>
<td>5′-TGT CCT CAT CCT CAC GGA GA-3′</td>
<td>310</td>
</tr>
<tr>
<td>A1 Reverse</td>
<td>586–605</td>
<td>5′-GCA CCC ACA GGA AGT TG-3′</td>
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</tr>
<tr>
<td>A2A Forward</td>
<td>1,488–1,511</td>
<td>5′-GTT CAG CTC CGG TCT CAA GAG CCA-3′</td>
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</tr>
<tr>
<td>A2A Reverse</td>
<td>1,705–1,728</td>
<td>5′-TGA GGA CAC TGC TGC TGC ATG CTG-3′</td>
<td>241</td>
</tr>
<tr>
<td>A2B Forward</td>
<td>699–723</td>
<td>5′-GCC TCG AGT GCT TTA CAG ACC GGC G-3′</td>
<td>175</td>
</tr>
<tr>
<td>A2B Reverse</td>
<td>848–873</td>
<td>5′-GAA AGT TGA CTG TCC GCC GGC CTG-3′</td>
<td></td>
</tr>
<tr>
<td>A3 Forward</td>
<td>132–152</td>
<td>5′-GAC CAC CAG CTA CTT TTT GA-3′</td>
<td>350</td>
</tr>
<tr>
<td>A3 Reverse</td>
<td>660–680</td>
<td>5′-GTC TTT AAC TCC CGA TGC-3′</td>
<td></td>
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Chemicals

CPA, CGS21680, NECA, DPCPX, enprofylline, MRS1523, ethanol, and DMSO were purchased from Sigma Chemical (St. Louis, MO). SCH58261 was a gift from Dr. A. Monopoli (Shearing Plough, Milan, Italy). CL-IB-MECA was obtained by SRI International from the National Institute of Mental Health Chemical Synthesis and Drug Supply Program.

Statistical Analysis

Experimental values are presented as means ± SE. EC100 was calculated. Analysis of variance was used to determine the level of significance among all groups. Pairs of groups were compared with unpaired two-tailed Student’s t-test. The P value for significance was set at 0.05.

RESULTS

Dose Responsiveness to Adenosine Receptor Agonists and the Effect of Corresponding Adenosine Receptor Antagonists

Aerosol challenge with NECA, a nonselective adenosine receptor agonist, elicited a dose-dependent increase in Penh in both ragweed-sensitized and -challenged and control mice. Bronchoconstrictor responsiveness to NECA was significantly enhanced in allergen-sensitized and -challenged mice compared with the controls. The maximum responsiveness to NECA (375 µg/ml) was 405.9 ± 36.6% in sensitized and challenged mice and 181.8 ± 7.7% in controls, respectively (P < 0.001). The EC100 was 30.66 µg/ml (95% confidence limits: 24.75–36.58) in ragweed-sensitized and -challenged mice and 142.03 µg/ml (95% confidence limits: 124.47–159.60) in control animals, respectively (P < 0.001, Fig. 1). The baseline Penh values between control (0.72 ± 0.06) and ragweed-sensitized and -challenged mice (0.78 ± 0.05) were not different. Vehicle (10% ethanol aerosolized for 2 min) used in experiment with agonists had no significant effect on Penh in both control and immunized mice. Also, 10% ethanol aerosolized for 5 min (vehicle for antagonists) was without an effect on basal bronchial tone.

Because NECA, like adenosine, has affinity for each of the four adenosine receptor subtypes (19), the question arises as to which adenosine receptor subtype is being activated in NECA-induced bronchoconstriction. To distinguish the subtype of adenosine receptors involved in NECA-induced bronchoconstrictor responsiveness, we employed selective adenosine agonists and antagonists. CPA is a potent and selective agonist at A1 receptors with reported inhibitor constant (Ki) values of 0.59, 460, and 240 nM at rat A1, A2A, and A3 receptors, respectively (11). Aerosol challenge of mice with CPA elicited little change in Penh in both control and immunized mice (Fig. 2A). Further experimentation was done to examine the bronchoconstrictor responsiveness to NECA with a selective A1 receptor antagonist, DPCPX, for which the Ki values at rat A1, A2A, and A3 receptors are 0.3 nM, 340 nM, and >10 µM, respectively (11). There was no effect of DPCPX on dose responsiveness to NECA observed after mice were pretreated with DPCPX (Fig. 2B). DPCPX per se had no effect on baseline Penh in both groups. Penh values before and after DPCPX under baseline conditions were 0.68 ± 0.03 and 0.62 ± 0.05 in control animals and 0.73 ± 0.06 and 0.67 ± 0.08 in sensitized and challenged mice, respectively (P > 0.05).

CGS21680 is the most potent, highly selective agonist at A2A receptors and is the ligand of choice for the characterization of A2A receptors. Aerosol challenge with CGS21680 did not affect the Penh (Fig. 3A). Pretreatment with an A2A receptor antagonist, SCH58261, a potent and highly selective A2A receptor antagonist both in vivo and in vitro (30), was unable to alter the bronchoconstrictor dose-response curve to NECA in either control or sensitized and challenged groups (Fig. 1).
There was no difference in baseline Penh value before and after SCH58261 in both groups of animals. The values were $0.64 \pm 0.04$ and $0.66 \pm 0.05$ (before vs. after SCH58261) in the control group and $0.74 \pm 0.04$ and $0.79 \pm 0.05$ (before vs. after SCH58261) in the sensitized and challenged groups, respectively.

CL-IB-MECA is a highly potent and selective A$_3$ receptor agonist with $K_i$ values of 820, 470, and 0.33 nM for rat A$_1$, A$_2A$, and A$_3$ receptors, respectively (11). CL-IB-MECA challenge produced marked bronchoconstrictor responsiveness in a dose-dependent manner (Fig. 4A). The maximum responsiveness to CL-IB-MECA (375 µg/ml) was $334.2 \pm 34.6\%$ in sensitized and challenged mice and $169 \pm 19.4\%$ in controls, respectively ($P < 0.001$). The EC$_{100}$ dose of CL-IB-MECA was $62.81 \mu$g/ml (95% confidence limits: 52.52–73.10) in ragweed-sensitized and -challenged mice and $158.55 \mu$g/ml (95% confidence limits: 114.59–200.21) in control animals, respectively. CL-IB-MECA-induced bronchoconstrictor responsiveness was completely inhibited by an A$_3$-selective antagonist, MRS1523, that is effective in various species (11) (Fig. 4A). MRS1523 itself did not affect basal bronchial tone. The basal Penh values before and after pretreatment with MRS1523 were $0.61 \pm 0.03$ and $0.72 \pm 0.09$ in control mice ($P > 0.05$) and in sensitized and challenged animals were $0.71 \pm 0.05$ and $0.64 \pm 0.07$ ($P > 0.05$), respectively. Vehicle alone (20% DMSO aerosolized for 2 or 5 min) had no effect on Penh in both control and allergen-sensitized and -challenged mice.

Furthermore, the effect of MRS1523 on a dose-response curve for NECA was also tested. It was found that NECA-induced bronchoconstriction was partially inhibited by MRS1523 (Fig. 4B), especially at the highest concentration (375 µg/ml of NECA). This suggests the involvement of other adenosine receptors, possibly the A$_2B$ receptors, in addition to A$_3$ receptors. Because there is a lack of availability of A$_2B$-selective antagonists commercially, enprofylline, which is somewhat selective for A$_2B$ adenosine receptors with $K_i$ values of 156, 32, 7, and 65 µM at human A$_1$, A$_2A$, A$_2B$, and A$_3$ receptors, respectively (10), was used to examine the effect of A$_2B$ receptors in NECA-induced bronchoconstrictor responsiveness. As expected, enprofylline did block NECA-induced bronchoconstriction as reflected by rightward shift of the dose-response curve (Fig. 5). EC$_{100}$ changed from $30.66 \mu$g/ml (95% confidence limits: 24.75–36.58) in sensitized and challenged mice to $157.40 \mu$g/ml (95% confidence limits: 114.59–200.21) after pretreatment with enprofylline in sensi-

**Fig. 2.** A: dose response to N$^\omega$-cyclopentyladenosine (CPA). B: dose response to NECA without and with pretreatment with 1,3-dipropyl-8-cyclopentylxanthine (DPCPX, $10^{-4}$ M). Con + DPCPX, control mice pretreated with DPCPX; Sen + DPCPX, sensitized mice pretreated with DPCPX; $n = 8–10$ for each group. Values are means ± SE. *$P < 0.05$, compared with Con group.

**Fig. 3.** A: dose response to 2-p-(2-carboxyethyl) phenethylamino-5’-N-ethylcarboxamidoadenosine (CGS21680). B: dose response to NECA without and with pretreatment with 7-(2-phenylethyl)-5-amino-2-(2-furyl)-pyrazolo-[4,3-e]-1,2,4-triazolo-[1,5-c]pyrimidine (SCH58261, $10^{-4}$ M). Con + SCH58261, control mice pretreated with SCH58261; Sen + SCH58261, sensitized mice pretreated with SCH58261; $n = 8–10$ for each group. Values are means ± SE. *$P < 0.05$, compared with Con group.
tized and challenged mice ($P < 0.01$). Also, a combination of enprofylline and MRS1523 completely inhibited the bronchoconstrictor responsiveness to NECA in both groups (Fig. 6), which was different from the effect of MRS1523 alone on a dose-response curve for NECA (Fig. 4B), suggesting the involvement of $A_{2B}$ and $A_3$ receptors in NECA-induced bronchoconstriction.

Adenosine Receptor Transcript Levels in Mouse Lungs

Adenosine receptor transcript levels were quantified in total RNA extracts isolated from whole lungs of both ragweed-sensitized and -challenged mice and control animals. RT-PCR revealed expression of four subtypes of adenosine receptors in the control lungs, with relatively high levels of $A_{2B}$ receptor message, intermediate levels of $A_1$ and $A_3$ receptor message, and low levels of $A_{2A}$ receptors (Fig. 7A). Mice sensitized and challenged with ragweed showed changes in the message for adenosine receptors in lungs. There were significant elevations in transcript levels of $A_{2B}$ and $A_3$ receptors after ragweed sensitization and challenge, which increased by 42.58% for $A_{2B}$ receptors ($P < 0.01$) and 29.05% for $A_3$ receptors ($P < 0.05$), respectively, and there was a decrease in the expression of $A_1$ adenosine receptors by 22.85% ($P < 0.05$). Little change in the expression of $A_{2A}$ receptors was observed (−4.39%) with a $P$ value $>0.05$ (Fig. 7B).

**DISCUSSION**

Consistent with our previous study (9), our present results show that NECA, a nonselective adenosine receptor agonist, causes bronchoconstriction, which is significantly greater in systemically allergen-sensitized, airway-challenged mice compared with control animals. Furthermore, the $A_1$ agonist CPA and $A_{2A}$
agonist CGS21680 produced little increase in Penh in this allergic mouse model. The selective A3 or A2A antagonist did not affect bronchoconstrictor responsiveness to NECA.

However, the A3 agonist CL-IB-MECA induced dose-dependent bronchoconstriction, which was completely inhibited by the selective A3 antagonist MRS1523 (Fig. 4A). At the same time, NECA-induced bronchoconstriction was partially inhibited by MRS1523, especially at the highest concentration of NECA, indicating the involvement of other adenosine receptors, possibly A2B receptors. To confirm our observations further, we employed a relatively selective A2B receptor antagonist, enprofylline, to study the effect of A2B receptors on NECA-induced bronchoconstriction. Enprofylline showed an attenuation of NECA-induced bronchoconstriction (Fig. 5). Because enprofylline is a relatively selective A2B antagonist with $K_i$ values of 7 and 65 $\mu$M for A2B and A3 receptors, respectively (10), it was still difficult to make conclusions about the role of A2B receptors from this experiment. Further supporting data were obtained using a combination of enprofylline with MRS1523, which showed that the partial inhibition of NECA-induced bronchoconstriction was completely blocked by a combination of enprofylline and MRS1523. These results were supported by the transcript levels of both A2B and A3 receptors being higher in the sensitized and challenged mice as opposed to control mice. Together, these results suggest that A2B and A3 adenosine receptors are possibly involved in NECA-induced bronchoconstriction in an allergic mouse model.

Adenosine and its agonist-induced bronchoconstriction have been studied in several animal models. A series of studies carried out by Pauwels and colleagues (31, 32) showed that BDE rats respond to adenosine agonists with the following order of potency: NECA showed that BDE rats respond to adenosine series of studies carried out by Pauwels and colleagues (31, 32) showed that BDE rats respond to adenosine series of studies carried out by Pauwels and colleagues (31, 32). In Brown Norway rats, Hannon and coworkers (13) suggested the involvement of A2B receptors in bronchoconstrictor responsiveness to adenosine. The same investigators, in a more recent study, questioned the role of A2B receptors in the bronchoconstrictor responsiveness to adenosine in Brown Norway rats (14). Studies using guinea pigs suggest the existence of a mechanism involving adenosine A3 receptors (18, 38). In another animal model, the animals immunized neonatally with ragweed or house dust mite developed bronchocencertror responsiveness to adenosine agonists (1). In this model, the order of agonist potency was CPA >> NECA >> CGS21680, and CPA-induced bronchoconstrictor responsiveness was blocked by a selective A1 antagonist, DPCPX (2). This pharmacological profile is typical of an adenosine A1 receptor subtype. In an extension of these studies, Nyce and Metzger (28) confirmed that an aerosolized phosphorothiate antisense oligodeoxynucleotide directed against the A1 receptor (but not the appropriate sense control) attenuated bronchoconstrictor responsiveness of immunized rabbits to either adenosine or adenosine agonists or dust mite allergen. To the best of our knowledge, the present study demonstrates in mice for the first time that adenosine- and its agonist-induced bronchoconstriction was most likely mediated by the activation of A2B and A3 adenosine receptors. Furthermore, an expression of all four subtypes of adenosine receptors was found in normal mouse lung using RT-PCR (Fig. 7A). Allergen sensitization and airway challenge produced a significant increase in the transcript levels of A2B and A3 adenosine receptors in mouse lung (Fig. 7B). Whether the effect of receptor activation in this model is a direct effect on airway smooth muscle or secondary to an effect on the activation of other cells (e.g., mast cells, eosinophils, neutrophils, lymphocytes, and macrophages) cannot be determined from this study. However, there is evidence that A2B adenosine receptors are expressed in human airway smooth muscle cells (26). Adenosine could directly affect airway smooth muscle cell contractility by modulating the
intracellular [Ca^{2+}] through the activation of adenosine A<sub>3</sub> receptors (25). Mice lacking the adenosine A<sub>3</sub> receptors showed attenuation of adenosine-induced bronchoconstriction (39). The A<sub>1</sub> receptors located directly on the smooth muscle in monkey and rabbit (27, 28) may play a role, since the selective depletion of the airway smooth muscle A<sub>1</sub> receptors by antisense reduced the bronchoconstrictor response to adenosine (28). However, our data showed a decrease in transcript level for A<sub>1</sub> receptors after allergen sensitization and challenge of mice, as well as the absence of CPA-induced bronchoconstriction in both control and sensitized and challenged mice. This discrepancy may be attributed to the use of a different animal model with varying pathophysiological conditions. A decrease in the expression of adenosine A<sub>1</sub> receptors has been reported in chronic inflammatory disease (16). Diminished A<sub>1</sub> receptor mRNA in mouse lung in our study may reflect a compensatory mechanism for the lung inflammation, since proinflammatory actions of adenosine A<sub>1</sub> receptor agonists on inflammatory cells have been suggested (7).

A variety of evidence points to the bronchoconstrictor responsiveness to adenosine being a consequence of activation of inflammatory cells in airways, especially mast cells and eosinophils (40, 41). It is well documented that A<sub>2A</sub>, A<sub>2B</sub>, and A<sub>3</sub> receptor mRNA is expressed in mouse bone marrow-derived mast cells (23, 35). In a functional study, Zhong et al. reported that enprofylline was able to protect ~30% of the adenosine-dependent mast cell degranulation in adenosine deaminase-deficient (ADA)-deficient mice (41). These authors further demonstrated with ADA-deficient and A<sub>3</sub> receptor double-knockout mice that A<sub>3</sub> receptors also played an important role in adenosine-mediated murine lung mast cell degranulation (42). Enhanced expression of adenosine A<sub>2B</sub> receptors on eosinophils has also been reported in the asthmatic lung (40). Our findings of increased expression of A<sub>2B</sub> and A<sub>3</sub> adenosine receptors in ragweed-sensitized and -challenged mouse lung was also confirmed by Chunn and colleagues in partially ADA-deficient mouse lung (6).

In summary, our data show an increased expression of A<sub>2B</sub> and A<sub>3</sub> receptor transcripts and no change in A<sub>2A</sub> receptor transcript with a decrease in A<sub>1</sub> message in sensitized and challenged mice. Although A<sub>1</sub>/A<sub>2A</sub> receptors were expressed in mouse lung, on the basis of Penh they seemed to play a little role in adenosine-mediated bronchoconstriction in our studies. Taking these data together, we conclude that adenosine A<sub>2B</sub> and A<sub>3</sub> receptors play an important role in adenosine-and its agonist-induced bronchoconstriction in our allergic mouse model.

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