Involvement of A1 adenosine receptors and neural pathways in adenosine-induced bronchoconstriction in mice

Xiaoyang Hua,1 Christopher J. Erikson,1 Kelly D. Chason,1 Craig N. Rosebrock,1 Deepak A. Deshpande,2 Raymond B. Penn,2 and Stephen L. Tilley1

1Department of Medicine, Division of Pulmonary and Critical Care Medicine, University of North Carolina at Chapel Hill, Chapel Hill, and 2Department of Internal Medicine, Center for Human Genomics, Wake Forest University, Winston-Salem, North Carolina

Submitted 12 February 2007; accepted in final form 18 April 2007

Adenosine is the cognate ligand for four different G protein-coupled receptors termed A1, A2A, A2B, and A3, and is a ubiquitous biological mediator involved in multiple physiological processes. Adenosine is believed to contribute to the pathogenesis of asthma and chronic obstructive pulmonary disease (COPD). Aerosolized adenosine can elicit bronchoconstriction in patients with asthma, COPD, and allergic rhinitis, but not in normal controls (9, 32, 35). Endogenous adenosine levels are significantly elevated in exhaled breath condensates and in bronchoalveolar lavage fluid collected from asthmatics (8, 10, 19). Therefore, adenosine receptors are attractive therapeutic targets for these diseases (21).

The detailed mechanisms mediating the bronchoconstrictor effects of adenosine have yet to be clarified. In most species, including humans, adenosine does not induce bronchoconstriction by directly activating receptors on airway smooth muscle. Rather, adenosine-induced bronchoconstriction occurs indirectly through mast cells and/or neural mechanisms (23, 28, 36). In asthmatic patients, premedication with mast cell membrane stabilizers or antagonism of mast cell autacoids can significantly diminish the bronchoconstriction induced by adenosine (33, 34). In the mouse, our group has previously shown that adenosine can directly stimulate mast cell degranulation in vivo and that mast cell activation accounts for a significant proportion of adenosine-induced airway responses observed in conscious animals (40, 41). However, it remains undetermined whether this airway responsiveness to adenosine, as measured by enhanced pause (Penh), reflects bronchoconstriction of the lower airways.

In addition to mast cells, neural pathways are also thought to be mechanistically involved in adenosine-induced bronchoconstriction. In asthmatic patients, the adenosine precursor AMP can induce bronchoconstriction, which is reduced by premedication with the anticholinergic ipratropium bromide (36). In guinea pigs, bronchoconstriction induced by the adenosine analog adenosine-5’-N-ethylcarboxamide (NECA) is attenuated by depletion of substance P, suggesting the participation of neuropeptide-containing nerves (26). Finally, attenuation of adenosine-induced bronchoconstriction by nedocromil sodium and sodium cromoglycate may occur through their effects on neuronal membranes rather than or in addition to effects on mast cell membranes. However, it remains unclear whether adenosine directly induces a neural reflex that causes bronchoconstriction or elicits neural activation in an indirect manner such as through mast cells.

The adenosine receptor subtype that mediates the bronchoconstrictor effect of adenosine is also an area of active investigation. Studies from human HMC-1 cell lines have shown that adenosine can activate these malignant mast cells via A2B receptors, and it has been suggested, based on these studies, that A2B receptors may be involved in adenosine-induced bronchoconstriction in asthmatics (12, 13). In this highly undifferentiated mast cell line, A2B receptors (which had previously been considered to couple exclusively to the Gq subunit) were found to activate Goq. This A2B activation of Goq was shown to stimulate PLC-γ/IP3/DAG signaling pathways and result in mast cell activation. However, studies to date have yet to show that such A2B-dependent mast cell activation occurs in mature human mast cells or mast cells from asthmatics. In contrast to HMC-1 cells, we have found in the mouse that A2B receptors mediate a nonredundant anti-inflammatory effect, inhibiting mast cell activation both in vitro and in vivo (18). Activation of mast cells by adenosine in the mouse occurs via the A3 receptor. By coupling to Gi/α subunit, A3 receptors stimulate calcium influx, resulting in the direct degranulation of mast cell granules.
of murine lung mast cells and skin mast cells, and potentiation of antigen-induced mast cell activity in murine bone marrow-derived mast cells (37, 40, 41, 43). A₁ adenosine receptors also activate the G_{i/o} subunit, which will trigger a PLC-IP₃/DAG signaling cascade (15). However, mast cells from most species (including mouse and human) do not express A₁ adenosine receptors. One exception is canine mastocytoma BR cell lines, but there is no evidence of a direct effect of this receptor on mast cell activation in vivo in dogs or most other species (4, 18, 43).

Several lines of evidence, however, suggest that A₁ receptors may play a role in adenosine-induced bronchoconstriction (2). Although Joaad and Kott (22) found no evidence of A₁ expression in human airways from nonasthmatic subjects, A₁ receptors are expressed at low levels in human airway smooth muscle (ASM) cultures (30); and, in ASM strips from asthmatics, A₁ receptor-mediated contraction has been reported (5). In rabbits, a role for the A₁ receptor in adenosine-induced bronchoconstriction is well established. Ali et al. (2, 3) have reported bronchial hyperresponsiveness to adenosine in an allergic rabbit model of asthma, with upregulation of A₁ receptors in ASM strips that were devoid of mast cells upon histological examination. A₁-mediated inositol 1,4,5-triphosphate generation in these same rabbits is further evidence of A₁ activation (1). On the basis of these observations, antisense oligodeoxynucleotides targeting A₁ expression has been prospectively proposed as one potential therapeutic strategy for asthma. Indeed, adenosine-induced bronchoconstriction is inhibited in a dust-mite model of allergic asthma in rabbits by aerosolized antisense oligodeoxynucleotides targeting the A₁ gene (31).

Collectively, these studies reflect a lack of consensus regarding the receptor subtypes, cells, and signaling mechanisms by which adenosine induces bronchoconstriction and suggest that more than one adenosine receptor may be involved. In this report, we employed a combination of physiological, pharmacological, and genetic approaches to define the mechanisms by which adenosine mediates bronchoconstriction in the mouse.

MATERIALS AND METHODS

Animals. All studies were conducted in accordance with and approved by the Institutional Animal Care and Use Committee guidelines of the University of North Carolina at Chapel Hill. C57BL/6 mice were purchased from the Jackson Laboratory. A₁⁻/⁻, A₂Δ⁻/⁻, A₂B⁻/⁻, and A₃⁻/⁻ mice were generated and genotyped by Southern Blot analysis or PCR as previously described (16, 18, 25, 37). A₁⁻/⁻, A₂Δ⁻/⁻, and A₃⁻/⁻ mice were backcrossed 12 generations to the C57BL/6 background. A₂B⁻/⁻ mice were backcrossed six generations to the C57BL/6 background. All mice were housed in a pathogen-free facility with 12-h day and night switch. For all experiments, mice were greater than 8 wk old, and wild-type and receptor-deficient animals were matched for age and sex.

Airway resistance in anesthetized mice. Mice were anesthetized with 90 mg/kg pentobarbital sodium, tracheostomized, and mechanically ventilated at a rate of 350 breaths/min, tidal volume 0.15 ml, and positive end-expiratory pressure 3–4 cmH₂O using a computer-controlled small animal ventilator (Sirec, Montreal, Canada) as previously described (39). Once ventilated, the right jugular vein was catheterized for adenosine delivery. After six measurements of basal lung resistance, 150 µl of adenosine (Sigma) in PBS (6 mg/ml) was infused over 5 s, and then lung resistance (R₁) was recorded every 10 s beginning immediately after adenosine infusion. The control animals received 150 µl of PBS only. In select experiments, the A₁ selective antagonist DPCPX (2 × 10⁻⁴ M) was delivered by aerosolization (5 min) or intraperitoneal injection (20 µl/gram body wt) 15 min before the physiological measurements. In some experiments, bilateral vagotomy was performed immediately following venous catheterization.

Airway responsiveness in conscious mice. Airway responsiveness in conscious mice was measured by a whole body plethysmograph (Buxco Electronics, Troy, NY) and expressed by the dimensionless index Penh as previously described (39). After 5 min of basal measurements, unrestrained mice were challenged by adenosine aerosol (6 mg/ml) for 5 min, and the Penh was monitored as indicated. In select experiments, mice were pretreated with aerosolized DPCPX (1 × 10⁻⁴ M, Sigma) or 0.75% bupivacaine (Abbott Laboratories, Chicago, IL) for 5–10 min or with an intraperitoneal injection of atropine sulfate (10 µmol/kg; American Pharmaceutical Partners, Los Angeles, CA) 2 min before adenosine aerosols. Tension development in tracheal ring preparations. Tracheal ring preparations and ex vivo tension measurements were performed as described previously (39). Briefly, mice were euthanized by inhalation of CO₂, and the trachea was rapidly removed and sectioned into 3- to 4-mm segments, which were then supported longitudinally by a Plexiglas rod in a glass organ bath containing Krebs-Henseleit solution maintained at 37°C. The upper support for the tracheal segment was secured by a silk thread loop, attached to a FT03 isometric transducer (Astra-Med, RI 02893), and force generation was recorded using an MP 100WS system (BIOPAC System). After initial stabilization in force measurements, ring tension was set to 0.5 g and maintained for 1 h. Carbachol (CCh), adenosine, or NECA was then added at increasing concentrations to establish dose-response curves. For subsequent analysis of the effect of adenosine on CCh-induced contraction, tissues were washed thoroughly, adjusted to the original resting tension, and contracted with the previously determined concentration of CCh producing 80% of the maximal contraction (EC₅₀). Upon obtaining a steady-state level of tension, adenosine or NECA was added to the bath progressively, and a level of steady-state tension was established for each concentration. At the conclusion of each experiment, tracheal segments were blotted on a gauze pad and weighed. Force generation was calculated as milligram of tension per milligram of tracheal ring weight.

Electronic field-stimulated contraction in isolated tracheal rings was performed as described previously (6, 42). Tracheal rings were mounted on a wire myograph chamber, and electrodes were placed perpendicular to the rings. Contractile responses were elicited by applying electrical field stimulations (EFS) using a Grass S444 stimulator (Grass Instruments). The rings were stimulated at 0.1, 0.3, 1, 10, and 30 Hz for 15 s each with a resting period of 2 min between stimulations. All the experiments were carried out at 90 V and 2-ms pulse duration, and buffer was changed periodically to maintain the ionic balance. A noncumulative frequency-response (S1) curve was established in all the rings, after which rings were allowed to recover for 45 min before being treated with vehicle or 1 µM NECA for 10 min. Following this treatment, a second noncumulative frequency-response (S2) curve was established using the frequencies and duration of stimulation described above. The contractile response to each stimulation was calculated by subtracting the basal tension from the peak tension developed after the EFS.

Statistical analysis. All data are presented as means ± SE. Paired Student’s t-test was used for comparison before and after treatment in the same subject; two-tailed, unequal Student’s t-test was used between different groups; repeated measures ANOVA was used to analyze differences between groups over time, from the beginning of adenosine aerosolization through the response period.
RESULTS

Adenosine-induced bronchoconstriction in anesthetized mice. Bronchoconstriction was evaluated by measuring \( R_L \) before and after adenosine infusions in anesthetized, tracheostomized, mechanically ventilated mice. After six measurements of basal resistance, 150 \( \mu \)l of adenosine in PBS (6 mg/ml) was infused through the catheterized right jugular vein over 5 s. \( R_L \) was measured immediately following adenosine infusions and then every 10 s for 4 min. In all adenosine-treated wild-type C57BL/6 mice, \( R_L \) increased immediately after the infusion and peaked within 20 s postchallenge before rapidly returning to baseline (Fig. 1A, real-time monitoring of \( R_L \); Fig. 1B, peak value within 30 s postchallenge). In contrast, \( R_L \) did not increase in vehicle-treated controls, suggesting that the rise in \( R_L \) immediately following intravenous adenosine was not due to the rapid expansion in blood volume as a result of the bolus. Figure 1C shows the effects of PBS, a pressure-volume (PV) loop, and adenosine, sequentially administered to C57BL/6 mice. As depicted, a slow, modest reduction in \( R_L \) occurs following PBS infusion. A more significant drop in \( R_L \) occurs, as expected, following deep inflation of the lungs during a PV loop. Adenosine bolus given after the PBS infusion and the PV loop resulted in a rapid rise in \( R_L \), similar to the effects shown in Fig. 1A. These data indicate that adenosine bolus injection is capable of eliciting a transient, reproducible, bronchoconstrictor response in anesthetized, paralyzed C57BL/6 mice.

Adenosine-induced bronchoconstriction in anesthetized mice is \( \text{A}_1 \) dependent. To determine the adenosine receptor subtype responsible for the bronchoconstrictor response observed in these deeply anesthetized mice, we examined the effects of intravenous adenosine on airway mechanics in mice lacking each adenosine receptor. As shown in Fig. 2A, adenosine infusion resulted in a statistically significant rise in \( R_L \) in mice lacking \( \text{A}_{2A}, \text{A}_{2B}, \text{A}_{3}, \) and \( \text{A}_1 \) receptors, which was similar in magnitude to that observed in wild-type animals. In contrast, adenosine-induced bronchoconstriction was absent in \( \text{A}_1 \)-/ mice. These data indicate that adenosine-induced bronchoconstriction in the anesthetized mouse is mediated by activation of \( \text{A}_1 \) receptors.

To further evaluate a role for the \( \text{A}_1 \) receptor in this adenosine-induced bronchoconstriction, we pretreated mice with \( \text{A}_1 \) selective antagonist (DPCPX) 15 min before adenosine infusion by either aerosolization or intraperitoneal injection. As shown in Fig. 2B, intraperitoneal injection, but not aerosolization, significantly reduced adenosine-induced bronchoconstriction. These data further demonstrate a role for \( \text{A}_1 \) adenosine receptors in adenosine-induced bronchoconstriction and suggest that the observed effect of intravenous delivery of adenosine is not due to the activation of \( \text{A}_1 \) adenosine receptors on epithelia or sensory nerve endings within the epithelium.

Effect of adenosine on ASM contraction ex vivo. To determine if \( \text{A}_1 \)-mediated bronchoconstriction in anesthetized mice resulted from a direct effect of adenosine on smooth muscle, we investigated ex vivo the effect of adenosine and NECA using isolated tracheal rings from C57BL/6 mice. Neither adenosine nor NECA, at concentrations ranging from \( 10^{-9} \) to \( 10^{-4} \) M, was able to generate tension in isolated mouse tracheal rings (Fig. 3, A and B), suggesting that \( \text{A}_1 \)-mediated bronchoconstriction in the anesthetized mouse occurs through an indirect manner.

Neural involvement in \( \text{A}_1 \)-mediated bronchoconstriction. To determine if \( \text{A}_1 \) receptor-mediated bronchoconstriction involves neural pathways, in vivo experiments were repeated following bilateral vagotomy. Successful vagotomy was confirmed by observing a reduction in \( R_L \) as a result of loss of cholinergic tone. Following six measurements of the new baseline \( R_L \), after vagotomy, adenosine (6 mg/ml) was infused through the jugular vein. Figure 4 shows that adenosine-induced bronchoconstriction is abolished following bilateral vagotomy, suggesting that adenosine-induced bronchoconstriction in anesthetized mice occurs indirectly through activation of cholinergic neural pathways.
in the anesthetized mouse, via the activation of A1 receptors on afferent nerves rather than direct actions on ASM or parasympathetic postganglionic fibers.

**A1 receptors are involved in adenosine-induced airway responsiveness in conscious mice.** Because anesthesia has been previously shown to blunt neural pathways, we examined airway responses to adenosine in conscious mice using whole body plethysmography. As shown in Fig. 6A, airway responsiveness to adenosine, as measured by Penh, was significantly attenuated in mice lacking the A1 receptor. A similar attenuation was observed in wild-type mice pretreated with the A1 antagonist DPCPX (Fig. 6B).

These data show that there is an A1-dependent and A1-independent component to adenosine’s actions in the airway of conscious mice.

**Neural pathways are involved in adenosine-induced airway responsiveness in conscious mice.** To investigate the role of neural pathways in adenosine-induced airway responsiveness in conscious mice, animals were pretreated with either an intraperitoneal injection of atropine or aerosolized bupivacaine, and then airway responsiveness to adenosine was measured by whole body plethysmography. Control animals were pretreated with vehicle. As shown in Fig. 7A, adenosine-induced airway responsiveness in mice pretreated with atropine was significantly reduced, compared with the groups pretreated with vehicle. In mice pretreated with bupivacaine, airway responsiveness to adenosine was also significantly attenuated.

**Effect of adenosine analog on precontracted ASM ex vivo.** There are at least three potential mechanisms by which adenosine might require cholinergic nerves to produce bronchoconstriction. First, adenosine could activate A1 receptors on afferent nerve endings, directly eliciting a neural reflex, subsequently contracting ASM. Second, A1 receptors on peripheral effector cells, such as smooth muscle cells, might potentiate the effect of spontaneously released acetylcholine from the efferent nerve ending. Third, adenosine could enhance postganglionic release of acetylcholine. To address these possibilities, we first investigated the effect of the adenosine analog NECA on mouse tracheal rings precontracted with the M3 receptor agonist CCh at submaximal dose, to determine whether CCh-induced contraction would be augmented. Instead of augmenting, NECA slightly inhibited CCh-induced contraction, suggesting that Gs-coupled A2A or A2B receptors are dominant on murine ASM, rather than Gi-coupled A1 receptors (Fig. 5A). Second, we assessed the effect of adenosine receptor activation on EFS on tracheal rings. As shown in Fig. 5B, prior addition of NECA had a minimal effect on an EFS-induced contraction of isolated tracheal rings, similar to that caused by prior addition of vehicle (DMSO) alone. These data support an indirect mechanism of adenosine-induced bronchoconstriction.
vagotomized and infused with adenosine (n = 8) or PBS (n = 6) was infused through the jugular vein, and RL was measured. Controls were not vagotomized and infused with adenosine (n = 11) or PBS (n = 9). **P < 0.001 by independent t-test vs. PBS-treated controls. ##P < 0.001 by independent t-test vs. adenosine-treated vagotomized group. Data represent the mean peak RL within 30 s post-challenge ± SE, presented as % of basal RL.

(Fig. 7B). These data are consistent with our findings in anesthetized animals, further supporting the existence of a cholinergic neural pathway in adenosine-induced bronchoconstriction in the mouse.

**DISCUSSION**

The mouse is an important experimental tool that has been widely used in the research related to adenosine and its receptors. However, it is still debatable whether adenosine can cause bronchoconstriction in this animal, because much of the previous research on adenosine’s influence on airway physiology relied exclusively on the derived dimensionless parameter, Penh. In this report, we include invasive methods in which airway mechanics are measured directly to assess the effect of adenosine on mouse lower airways. Bolus injection of adenosine produced an immediate increase in resistance in anesthetized C57BL/6 mice. These transient constrictor responses could also be elicited in A2A−/−, A2B−/−, and A3−/− mice, but were abolished by either targeted deletion of A1 gene or bilateral vagotomy, suggesting that adenosine is capable of eliciting bronchoconstriction in anesthetized C57BL/6 mice through an A1- and vagus nerve-dependent mechanism.

Our findings are the first to demonstrate a role for A1 receptors in adenosine-induced bronchoconstriction in mice. These data are consistent with several previous reports in other species. Ali et al. (2) showed that adenosine-induced bronchoconstriction in an allergic rabbit model can be blocked by A1 selective antagonism. A later report by Nyce et al. (31) using this same model showed that pretreatment with an antisense oligodeoxynucleotide targeting the A1 gene could also block adenosine’s effect on airway tone. Additionally, a recent report in allergic guinea pigs showed that adenosine-induced bronchoconstriction was also attenuated by A1 selective antagonism (23). Collectively, these studies suggest the utility of targeting A1 receptors in asthma, an idea currently being tested in clinical trials (38).

The mechanisms by which activation of A1 receptors induce bronchoconstriction is controversial. In allergic rabbits, adenosine’s contractile effect on the airway occurs through activation of A1 receptors on ASM cells (2). In human asthmatics, however, adenosine-induced bronchoconstriction is believed to occur through indirect pathways. In our studies in the mouse, treatment with adenosine or its potent analog NECA could not elicit a contractile response in isolated tracheal rings, suggesting that A1-mediated bronchoconstriction in vivo is not a direct effect of adenosine on ASM cells. A recent study using cultured human smooth muscle cells showed that A1, but not A2A or A3 selective agonists, could elicit an immediate calcium influx, suggesting that activation of A1 might potentiate the responsiveness of ASM cells to acetylcholine in vivo (11). Hence, the absence of adenosine’s contractile effect on isolated tracheal rings of mice could result from the loss of cholinergic innervation. However, our ex vivo data demonstrate that adenosine cannot augment, but rather slightly inhibits, the contractile effect of CCh on tracheal rings, further suggesting that A1-mediated bronchoconstriction in vivo is not a result of cooperativity between A1 receptors and M3 muscarinic receptors on ASM cells. Moreover, our data also fail to show a synergistic effect of NECA on EFS-induced ASM contraction ex vivo, indicating that adenosine does not enhance...
acetylcholine release from parasympathetic postganglionic synapses.

While a mast cell-dependent component to adenosine-induced bronchoconstriction has been reported in multiple species (5, 28, 34, 40), the A1-dependent bronchoconstriction observed in anesthetized mice appears to be mast cell independent, as mast cells from most species, including mouse, do not express A1 receptors (14, 18, 37, 43); and, A3 receptor activation has been shown to account for the entirety of mast cell degranulation in the murine lung following exposure to adenosine (40).

In the current study, adenosine-induced bronchoconstriction in anesthetized mice was abolished by either bilateral vagotomy or targeted disruption of the A1 gene. Additionally, adenosine-induced airway responsiveness in unrestrained, conscious mice was attenuated by A1 receptor deficiency or antagonism and by inhibition of cholinergic pathways. These data suggest a link between A1 receptors and neural reflexes in adenosine-induced bronchoconstriction in mice. Reflex pathways involving sensory vagal C-fibers have been reported for several cardiopulmonary responses to adenosine (24, 29). In anesthetized rats, the right atrial injection of adenosine activated 68% of vagal afferent pulmonary C-fibers, and this stimulatory effect of adenosine was completely prevented by pretreatment with the A1 receptor antagonist DPCPX (17). Similar experiments have shown that adenosine can evoke action potential discharges in vagal C-fibers in guinea pig lungs (7). Supporting these functional studies, radioreceptor assays, in situ hybridization, and immunocytochemistry have demonstrated the presence of A1 adenosine receptors on vagal afferent neurons (29).

Previously we have demonstrated that adenosine can activate airway mast cells and modulate airway physiology in conscious mice through A3 receptors (40). In the current study, however, we did not observe any A3-dependent component of adenosine-induced bronchoconstriction in the anesthetized and mechanically ventilated mouse. There are at least two possible explanations for these observations. First, the effect of mast cell activation on airway tone is diminished by anesthesia. In anesthetized and mechanically ventilated mice, the intravenous administration of mast cell autacoids, including histamine, prostaglandin, and leukotrienes, does not elicit bronchoconstriction, suggesting that mast cell autacoids in the mouse cannot cause direct contraction of ASM nor elicit a neural reflex to cause bronchoconstriction indirectly (whereas intravenous adenosine does) (26a). Martin et al. (27) reported that mast cell degranulation at modest magnitude (a magnitude...
similar to adenosine-induced mast cell degranulation) could potentiate the response of ASM to muscarinic agonists in anesthetized mice, suggesting that mast cell degranulation by adenosine in mice might enhance the effect of vagal activity on airway tone. Thus it is possible that the absence of bronchoconstriction by adenosine-driven mast cell degranulation in anesthetized mice could be the result of reduced parasympathetic tone under deep anesthesia (20). Another explanation is that adenosine-induced mast cell degranulation is insufficient to produce bronchoconstriction in mice. However, because of the unavailability of definitive methodology to measure bronchoconstriction in conscious mice and the widely inhibitory effect of anesthesia on a number of physiological responses, this conclusion is yet to be confirmed. In the current study, although we cannot demonstrate conclusively that the rise in Pern induced by adenosine reflects bronchoconstriction, we did observe that these effects of adenosine in conscious mice were almost abolished by local administration of anesthetic. These results suggest that adenosine-induced airway responsiveness in conscious mice, through activation of both A1 receptors on neurons and A3 receptors on mast cells, both require neural activity.

Adenosine-induced bronchoconstriction is a complex physiological process, involving multiple cell types and multiple adenosine receptors. In this report, we present data implicating the A1 adenosine receptor and cholinergic neural pathways in adenosine-induced bronchoconstriction in the naïve mouse. Our findings also suggest an A1-independent component in conscious animals, which is consistent with previous work showing an A3-dependent, mast cell-dependent, adenosine-induced airway responsiveness in the conscious mouse. Although the methodology to measure definite bronchoconstriction in conscious mice is lacking, collectively, these studies suggest a model in which activation of A1 receptors on neurons and A3 receptors on mast cells act together to mediate the full effect of adenosine on the murine airway. Since these studies were performed in naïve mice, pathways may or may not be similar in inflamed airways on mice or asthmatics. However, studies in asthmatics support a role for both mast cells and neurons in adenosine-induced bronchoconstriction in humans, suggesting that further studies on the murine airway, particularly in models of asthma, may be useful for further dissection of the mechanistic pathways of adenosine-induced bronchoconstriction and the mast cell-neuronal axis in the lung.

ACKNOWLEDGMENTS

We thank the Keck family for generous support of the Keck Animal Models Facility at the University of North Carolina and Yvonne Brooks for assistance with mouse genotyping. We also thank Dr. Bertil Fredholm and Dr. Catherine Ledent for providing the A1- and A2A-deficient mice, respectively, which were used to generate the congenic C57BL/6 mice used in these studies.

GRANTS

This work was supported by National Heart, Lung, and Blood Institute Grants HL-071802 (S. L. Tilley) and HL-58506 (R. B. Penn). D. A. Deshpande is supported by the Pathway to Independence Award K99-HL-087560.

REFERENCES


