Receptors and pathways mediating the effects of prostaglandin E₂ on airway tone

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Shorty after its discovery in the 1960s, prostaglandin E₂ (PGE₂) was observed to have potent relaxant effects on airway smooth muscle, making this endogenous lipid an attractive candidate drug for asthma (18, 34). However, human studies with aerosolized PGE₂ have demonstrated inconsistent effects on airway tone, with most asthmatics showing a bronchodilator response but some developing profound bronchoconstriction requiring beta agonist rescue (6, 19). Ex vivo studies from humans and animals have also shown that PGE₂ can both relax and constrict airway smooth muscle, a paradox explained when it was recognized that PGE₂ could act through more than one receptor (9, 20, 26).

Four distinct cell surface receptors for PGE₂ (E-prostanoid (EP)) have been identified and cloned, each with unique signal transduction mechanisms as a result of coupling to different G proteins (23). Activation of one or a combination of receptors, each of which may be differentially expressed on airway smooth muscle, epithelia, neurons, and immune effector cells, results in a specific physiological response. EP2 and EP4 receptors couple to Gs, and activation of these receptors results in stimulation of adenyl cyclase and increases in intracellular cAMP. Various EP3 isoforms, arising from the differential splicing of the EP3 gene, have been identified both in humans and in mice (1, 24).

Differences in the cytoplasmic domain of the EP3 receptor leads to the activation of different intracellular pathways, depending on which isoform the cell expresses (22). In both mouse and human cells, expression of isoforms capable of coupling to Gs, Gi, and Gq have been described (15, 24). Activation of the EP1 receptor leads to an increase in intracellular calcium. Expression analysis has detected mRNA corresponding to all four receptors in the mouse lung (16, 36).

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Although EP receptor distribution among cells within the lung is not well established, it is clear that various airway cells can express multiple EP receptors. Thus the complexity of the integrated airway response to PGE2 (altered bronchomotor tone) is a function of cooperativity among various EP receptors in multiple cell types in the airway. To clarify the relative contributions of each EP receptor and the integrative nature of EP receptor signaling in the airway, we examined airway reactivity in response to PGE2 in mice deficient in each of the PGE2 receptors using a number of different methodologies.

MATERIALS AND METHODS

Experimental animals. All studies were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals as well as the Institutional Animal Care and Use Committee guidelines of the University of North Carolina at Chapel Hill. Wild-type C57BL/6, 129X1/Sv, DBA/1, DBA/2, and BALB/c mice were purchased from Taconic. Mice deficient in the EP1, EP2, EP3, and EP4 receptors were generated and genotyped by Southern blot analysis or PCR as previously described (7, 25, 32, 35). EP1-, EP2-, and EP3-deficient mice were backcrossed 12 generations to the C57BL/6 background. EP4+/− mice on inbred backgrounds, including C57BL/6, die from patent ductus arteriosus but survive on a mixed genetic background consisting of DBA/2, C57BL/6, and 129/Ola. A recombinant inbred strain consisting of these backgrounds has been developed to carry the EP4 mutation and EP4+/+ controls. EP1 mice on an inbred DBA/1 background were also used for some experiments where indicated. For all experiments, mice were 8–16 wk old, and wild-type and receptor-deficient animals were matched for age, sex, and strain.

Measurements of airway responsiveness in conscious mice. Mice were placed in a whole body plethysmograph (Buxco Electronics, Troy, NY), and baseline measurements of enhanced pause (Penh) were obtained. Penh is a dimensionless index calculated from inspiratory pressures, expiratory pressures, and expiratory time and has been shown to correlate with direct measures of pulmonary resistance in mechanically ventilated animals (11). Penh was then measured in response to aerosols of vehicle (10% ethanol), PGE2 (100 μg/ml), or methacholine (100 μg/ml). In some experiments, mice were treated with aerosolized 0.75% bupivacaine (Abbott Laboratories, Chicago, IL) for 10 min or an intraperitoneal injection of 10 μmol/kg atropine sulfate (American Pharmaceutical Partners, Los Angeles, CA) 2 min before PGE2 aerosols.

Measurements of airway resistance in anesthetized mice. Mice were anesthetized with 70–90 mg/kg pentobarbital sodium, tracheostomized, and mechanically ventilated at a rate of 300 breaths/min, tidal volume of 6 cc/kg, and positive end-expiratory pressure of 3–4 cmH2O with a computer-controlled small-animal ventilator (Sireq, Montreal, Canada). Once ventilated, mice were paralyzed with 0.8 mg/kg pancuronium bromide, and we determined lung resistance by transducing airway pressures and using the equation of motion. Aerosols of PGE2 (Cayman Chemical, Ann Arbor, MI) and methacholine (Sigma Chemical, St. Louis, MO) were delivered via nebulizer (DeVilbiss) through a side port in the ventilator circuit. Indomethacin-treated mice received an intravenous tail vein injection of 10 mg/kg indomethacin within 4 h of the experiment.

Tension development in tracheal ring preparations. Mice were euthanized by inhalation of CO2, the trachea was rapidly excised, and serosa and connective tissue were removed. Each trachea was sectioned into 3- to 4-mm segments, rinsed, and incubated for 30 min in Ham’s F-12 and DMEM (vol/vol) media containing 5,958 mg/l HEPES, 10% FBS, 584 mg/l glutamine, and 0.2g/l CaCl2. Segments were then supported longitudinally by a Plexiglas rod with a stainless steel pin in a double-jacketed, glass organ bath perfused with 10 ml of Krebs-Henseleit solution maintained at 37°C (2, 14). The bathing solution was continuously aerated with 5% CO2 and 95% O2 at a pH of 7.40–7.45 for the duration of the experiment. The upper support for the tracheal segment was secured by a silk thread loop attached to a FT03 isometric transducer (Astro-Med, West Warwick, RI), and force generation was recorded with a MP 100WS system (BIOPAC Systems). After initial stabilization in force measurements, ring tension was set to 0.5 g and maintained for 1 h. Carbachol (CCh) or PGE2 was then added at increasing concentrations to establish dose-response curves. Concentrations were increased only when force generation responses to the previous dose had stabilized. IC50 values and maximal relaxation effect of PGE2 (following assessment of the dose-dependent response to CCh), tissues were washed thoroughly, adjusted to the original resting tension, and contracted with the previously determined concentration of CCh producing 80% of the maximal concentration. Upon obtaining a steady-state level of tension, we added PGE2 (0.01–10 μM) to the bath progressively, and a level of steady-state tension was established for each concentration. IC50 values and maximal relaxation effect (expressed as a percentage of maximal inhibition induced by 200 μM papaverine added at the end of PGE2 dose-response determination) were calculated and compared among groups. At the conclusion of each experiment, tracheal segments were blotted on a gauze pad and weighed. Force generation was calculated as milligrams of tension per milligram of tracheal ring weight.

Statistical analysis. All data are presented as means ± SE. Statistical significance was assessed by the two-tailed, unequal Student’s t-test. The two-factor ANOVA with replication was used to analyze differences between groups over time from the beginning of PGE2 aerosolization through the response period.

RESULTS

Effect of PGE2 on the airways of conscious mice. To determine the effects of PGE2 on the airways of conscious mice, we evaluated six different mouse strains using whole body plethysmography. Mice were exposed to aerosolized PGE2 (100 μg/ml) for 5 min following a 5-min aerosolization of 10% ethanol vehicle (Fig. 1). A small but statistically significant increase in Penh in response to ethanol vehicle was seen in BALB/c and C57BL/6 animals. PGE2 had no effect above that seen with vehicle alone in 129X1/Sv, 129SvEV, and DBA/2 mice. A trend toward responsiveness to PGE2 (P = 0.13) was seen in DBA/1 mice, whereas statistically significant increases in Penh above that produced by vehicle were seen in BALB/c and C57BL/6 animals.

PGE2-induced airway responses are diminished in EP1- and EP3-deficient mice. To determine the receptors mediating these effects of PGE2, we measured...
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before, during, or following PGE<sub>2</sub> aerosol (Fig. 2, B and D). In contrast, the response to PGE<sub>2</sub> was significantly attenuated in EP1<sup>−/−</sup> and EP3<sup>−/−</sup> animals, suggesting that both receptors contribute to PGE<sub>2</sub>-induced airway responsiveness in C57BL/6 mice (Fig. 2, A and C). To confirm that these effects were mediated by a loss of EP1 and EP3 receptor signaling rather than by altered intrinsic contractile properties, we measured the response to methacholine in EP1<sup>−/−</sup>, EP3<sup>−/−</sup>, and wild-type control animals. No differences were observed between EP1<sup>−/−</sup> or EP3<sup>−/−</sup> mice and their respective wild-type controls following exposure to 100 mg/ml methacholine for 5 min (data not shown).

To further evaluate the role of the EP1 receptor in this response, we examined mice from the original DBA/1 line on which this mutation was generated. In contrast to our findings with C57BL/6 mice, EP1<sup>−/−</sup> mice on the DBA/1 background showed no response to PGE<sub>2</sub>, whereas DBA/1 wild-type control mice showed significant increases in Penh following PGE<sub>2</sub> exposure (Fig. 3).

**Effect of PGE<sub>2</sub> on lung resistance in anesthetized mice.** To further evaluate the effects of PGE<sub>2</sub> on bronchomotor tone in vivo, we measured lung resistance before and after aerosols of PGE<sub>2</sub> in anesthetized, tracheostomized, mechanically ventilated mice. Surprisingly, no change in resistance was observed following exposure of the airways of C57BL/6 or DBA/1lac wild-type mice to high concentrations of PGE<sub>2</sub> aerosols (100 μg/ml) (Fig. 4). To ensure that the failure to observe a constrictor response was not due to opposing actions of the EP2 receptor, we repeated this experiment with EP2<sup>−/−</sup> mice. PGE<sub>2</sub> failed to alter baseline airway tone in this mutant mouse line as well (data not shown). Because endogenously released prostanoids may activate receptors on airway smooth muscle, po-

Fig. 1. Airway responsiveness to PGE<sub>2</sub> among different inbred mouse strains. Airway responsiveness was measured by whole body plethysmography at baseline after a 5-min exposure to aerosolized vehicle (10% ethanol in PBS) and after a 5-min exposure to aerosolized PGE<sub>2</sub> (100 μg/ml). Data represent the mean enhanced pause (Penh) during the 5-min baseline period (open bars) and mean Penh during the 2-min period immediately following vehicle (hatched bars) or PGE<sub>2</sub> (solid bars) aerosolization ± SE, n = 8 per group. *P < 0.05 compared with vehicle; +P < 0.05 compared with baseline.

airway responsiveness following PGE<sub>2</sub> exposure in EP receptor-deficient mice (Fig. 2). In all cases except for EP4, we carried out the experiments using mice in which the mutant allele was introduced onto the inbred C57BL/6 genetic background. In the case of EP4 mice, a recombinant inbred strain, as well as the corresponding congenic EP4<sup>−/−</sup> line, was used. This strain allows EP4<sup>−/−</sup> animals to undergo closure of the ductus arteriosus in the absence of the receptor and thus survive to adulthood (25). No significant differences were seen between EP2<sup>−/−</sup> or EP4<sup>−/−</sup> mice and their respective wild-type controls at any time point

![Fig. 2. Airway responsiveness to PGE<sub>2</sub> in PGE<sub>2</sub> (EP) receptor-deficient mice. After a 5-min exposure to aerosolized vehicle, airway responsiveness was measured by whole body plethysmography during and after exposure to a 5-min aerosol of PGE<sub>2</sub> (100 μg/ml). Data represent mean Penh each min during (1–5) and following (6–12) PGE<sub>2</sub> exposure ± SE. • Wild-type controls (+/+); ◦ EP-deficient mice (−/−). A: EP1<sup>+/+</sup> n = 20, EP1<sup>−/−</sup> n = 20. B: EP2<sup>+/+</sup> n = 21, EP2<sup>−/−</sup> n = 18. C: EP3<sup>+/+</sup> n = 28, EP3<sup>−/−</sup> n = 30. D: EP4<sup>+/+</sup> n = 10, EP4<sup>−/−</sup> n = 9. *P < 0.05; **P < 0.005. Airway responsiveness in EP1<sup>−/−</sup> and EP3<sup>−/−</sup> mice from the beginning of PGE<sub>2</sub> aerosol until the end of the experiment was significantly different (P < 0.001) compared with their respective wild-type controls by 2-factor ANOVA. All animals were on a C57BL/6 background, except EP4<sup>−/−</sup> mice and their wild-type controls, which were on a recombinant inbred strain.

![Fig. 2](http://ajplung.physiology.org/)

**Effect of PGE<sub>2</sub> on lung resistance in anesthetized mice.** To further evaluate the effects of PGE<sub>2</sub> on bronchomotor tone in vivo, we measured lung resistance before and after aerosols of PGE<sub>2</sub> in anesthetized, tracheostomized, mechanically ventilated mice. Surprisingly, no change in resistance was observed following exposure of the airways of C57BL/6 or DBA/1lac wild-type mice to high concentrations of PGE<sub>2</sub> aerosols (100 μg/ml) (Fig. 4). To ensure that the failure to observe a constrictor response was not due to opposing actions of the EP2 receptor, we repeated this experiment with EP2<sup>−/−</sup> mice. PGE<sub>2</sub> failed to alter baseline airway tone in this mutant mouse line as well (data not shown). Because endogenously released prostanoids may activate receptors on airway smooth muscle, po-
tentially rendering them refractory to exogenously administered PGE2, we repeated measurements of lung resistance in response to aerosolized PGE2 in mice pretreated with indomethacin. Again, no changes in lung resistance were observed following PGE2 exposure (data not shown).

In contrast, the bronchoprotective actions of PGE2 were easily measured in this system. As shown in Fig. 5, pretreatment of wild-type mice with PGE2 dramatically attenuated the bronchoconstrictor response to methacholine. This protective response was partially lost in mice lacking the Gs-coupled EP2 receptor (Fig. 5). Because some bronchoprotection remained in the EP2−/− animals, we hypothesized that the Gs-coupled EP4 receptor may also play a role. However, methacholine-induced constriction was attenuated by PGE2 to a similar degree in EP4−/− and +/+ mice, suggesting that the EP4 receptor does not contribute to this bronchoprotective response (data not shown).

Effect of PGE2 on ex vivo tracheal rings. To begin to define the cell types through which PGE2 influences bronchomotor tone, we examined its effects on isolated tracheal rings from each of the four mouse lines and the appropriate wild-type controls. In contrast to the airway response produced in conscious mice but similar to our findings in anesthetized animals, PGE2 failed to elicit contraction of tracheal rings under spontaneous tone (Fig. 6). This was the case even in tracheal rings from EP2−/− mice, where potential antagonism of contraction by EP2 receptor activation would be absent (data not shown). To exclude the possibility that the handling of the tissue resulted in production of PGE2 and receptor desensitization, as well as to eliminate any spontaneous tone produced by other prostanoids, we carried out a similar series of experiments in the presence of the potent cyclooxygenase inhibitor indomethacin. Again, no contraction of the tracheal rings was observed in response to PGE2 in concentrations ranging from 1 × 10−11 to 5 × 10−5 M (Fig. 6).

Although no contractile effects of PGE2 could be identified in this system, the relaxant actions of PGE2 were easily measured in rings preconstricted by the cholinergic receptor agonist CCh and assigned in part to specific EP receptors. As shown in Fig. 7, PGE2 produced dose-dependent relaxation of EP1−/−, EP3−/−, and EP4−/− rings that was no different from their respective wild-type controls. Interestingly, the effectiveness of PGE2 in relaxing the constricted airway differed between the two strains of control mice, with the C57BL/6 rings relaxing to ~15% and the
recombinant inbred strain (EP4+/+ controls) relaxing to ~50% of maximal CCh-induced tension, respectively. These relaxant actions of PGE2 are unlikely to be due to activation of EP receptors on epithelial cells and subsequent production of factors by these cells, as the relaxation response persisted in strips in which the epithelia had been denuded (data not shown). Enhanced relaxation was not observed in tracheal rings lacking either EP1 or EP3 receptors, as might be expected if the relaxant actions of PGE2 on preconstricted airways were limited by activation of a constricting EP receptor on airway smooth muscle or epithelia.

By contrast, the loss of the EP2 receptor almost completely eliminated the relaxation response induced by PGE2 in tracheal rings (Fig. 7B), indicating that the airway smooth muscle relaxant actions of PGE2 are highly dependent on the EP2 receptor. A small residual relaxation response was noted in EP2−/− rings, suggesting that a second receptor may contribute modestly to PGE2-induced relaxation in the absence of EP2. However, when EP2 is present, a contribution of a second relaxant receptor could not be detected in rings from any of the other EP-deficient lines.

Airway responses in conscious mice are prevented by interruption of neural pathways. Because all receptors for PGE2 are expressed on sensory neurons, we considered the possibility that the airway response to PGE2 observed in conscious animals could be neurally mediated and, consequently, more difficult to measure in isolated tissue or anesthetized mice. Thus to determine whether the physiological changes observed in conscious mice were mediated by activation of neural pathways, we measured airway responsiveness to PGE2 following treatment with the local anesthetic bupivacaine. As shown in Fig. 8A airway responsiveness failed to increase following PGE2 exposure in mice pretreated with bupivacaine (P<0.05). To test the contribution of the parasympathetic nervous system to this response, we measured PGE2-induced airway responsiveness following pretreatment with the anticholinergic agent atropine. Similar to bupivacaine, atrpine prevented increases in Penh following PGE2 aerosols (Fig. 8B). Together, these data suggest that PGE2 can increase airway responsiveness in conscious mice by activating EP receptors on nerves and that this action is mediated primarily by cholinergic pathways.

DISCUSSION

Although a number of previous studies have examined the bronchodilatory effects of PGE2, less information is available concerning the bronchoconstrictive pathways that are in part responsible for the failure to...
even with high levels of PGE2 or in mice lacking the thetized, tracheostomized mouse or in excised tissue, P described above. * following an injection of atropine (10 mmol/kg ip, \( n = 10 \)) PGE2 exposure. B: following an injection of atropine (10 mmol/kg ip, \( n = 12 \)) PGE2 exposure. Bupivacaine blocks both sensory and motor nerves at the doses we administered, and airway responsiveness to PGE2 was prevented by bupivacaine pretreatment. To further define the mechanism by which PGE2 produces these physiological changes in conscious mice, we blocked cholinergic neurotransmission by pretreating animals with atropine, an antagonist of muscarinic acetylcholine receptors. This action eliminated airway responsiveness to PGE2, implicating reflex cholinergic bronchoconstriction in this process.

Our results reveal that the EP1 and the EP3 receptors are responsible for mediating these reflex cholinergic effects of PGE2 on the airway. This is consistent with the expression of both EP1 and EP3 by sensory nerves and the demonstrated role for both the EP1 and EP3 receptors in transmitting PGE2-mediated pain responses in vivo. Our results therefore suggest that the ability of PGE2 to both constrict and dilate airways is not related to differential activation of procontractile and prorelaxant EP receptors on airway smooth muscle. Although both EP1 and EP3 receptors, when activated, can increase intracellular calcium and thus, if functioning on smooth muscle cells, could mediate bronchoconstriction, no evidence for the presence of such a pathway was obtained. Rather, we suggest a model in which the ability of PGE2 to differentially affect the airway is the result of the actions of PGE2 on two different physiological systems, its ability to modulate directly the activity of the airway smooth muscle via EP2, and the ability to stimulate either directly or indirectly the activity of the parasympathetic neurons of the respiratory tract via EP1/EP3.
Interestingly, we observed that several of the effects of PGE₂ on the airway differed among mouse strains. For example, although a constrictor response to PGE₂ was easily seen in C57BL/6, DBA/1, and BALB/c mice, it was much more difficult to detect in other mouse strains. In fact, PGE₂ had no detectable effect on the airways of 129X1/Sv mice. It is interesting to speculate that the underlying reason for this variation in the responsiveness of various mouse strains may be similar to variability seen in humans following exposure to PGE₂ aerosols. In addition to quantitative differences, the background strain of mice also influenced the PGE₂ receptors contributing to the response. Although loss of the EP1 receptor completely abolished the airway response in DBA/1 mice, loss of this receptor in C57BL/6 mice only attenuated the response. This finding can be explained by the fact that an attenuated response was seen in the EP3/−/− mice on this background. These results raise the possibility that differential utilization of EP receptors might represent a fundamental difference between inbred mouse strains and contribute to the variation observed between strains in many of the physiological processes where prostaglandins play a role.

Despite the potent bronchodilatory and bronchoprotective effects of PGE₂, this prostaglandin has never been exploited as a therapy for asthma in part because of its capacity to provoke bronchoconstriction in a subset of asthmatics. In addition, PGE aerosols are irritative to human airways, producing cough and retrosternal burning. A number of lines of evidence suggest that, similar to the action of PGE₂ that we describe here, these effects of PGE₂ are neurally mediated. First, it has been shown that PGE₂ can stimulate intrapulmonary C fibers and to a lesser extent rapidly adapting irritant receptors (5). Consistent with this observation is the activation of afferent vagal C fibers by both intravenous and endobronchial administration of PGE₂ to dogs (29). Our study in the mouse for the first time shows that the potentially clinically beneficial actions of PGE₂ are mediated by a different PGE₂ receptor than the potentially dangerous bronchoconstrictive actions. If a parallel situation can be demonstrated in humans, it would suggest that EP2 receptor-specific agonists might provide the valuable bronchodilatory agent that was envisioned over twenty years ago when trials were initiated to examine the usefulness of PGE₂ for the treatment of asthma.

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REFERENCES
4. Coleman RA and Kennedy I. Characterisation of the prosta-

5. Coleridge HM, Coleridge JC, Ginzel BH, Baker DG, Ban-
9. Gardiner PJ and Collier HO. Specific receptors for prosta-
10. Geppert EP and Boushey HA. An investigation of the mecha-
glandin E receptor EP2 subtype: cloning, expression, and north-
17. Lammers JW, Minette P, McCusker MT, Chung KF, and Barnes PJ. Nonadrenergic bronchodilator mechanisms in nor-
18. Main IHM. The inhibitory actions of prostaglandins on respira-

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