EP2 receptor mediates bronchodilation by PGE2 in mice

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Sheller, J. R., Daphne Mitchell, Barbara Meyrick, John Oates, and Richard Breyer. EP2 receptor mediates bronchodilation by PGE2 in mice. J Appl Physiol 88: 2214–2218, 2000.—PGE2 is an important cyclooxygenase product that modulates airway inflammatory and smooth muscle responses. Signal transduction is mediated by four EP receptor subtypes that cause distinct effects on cell metabolism. To determine the role of EP2 receptor activation, we produced a mouse lacking the EP2 receptor by targeted gene disruption. The effect of aerosolized PGE2 and other agonists was measured using barometric plethysmography and by measurements of lung resistance in mechanically ventilated mice. Inhalation of PGE2 inhibited methacholine responses in wild-type but not in mice lacking the EP2 receptor [EP2(−/−)]. After airway constriction was induced by methacholine aerosol, PGE2 reduced the airway constriction enhanced pause in wild-type mice (from 0.88 ± 0.15 to 0.55 ± 0.06) but increased it in EP2(−/−) mice (from 0.73 ± 0.08 to 1.27 ± 0.19). Similar results were obtained in mechanically ventilated mice. These data indicate that the EP2 receptor mediates the bronchodilation effect of PGE2.

pharmacology; airway reactivity; methacholine; knockout mice

PROSTAGLANDIN E2 (PGE2) is an endogenous lipid autacoid derived from the action of the cyclooxygenase enzymes on free arachidonic acid and subsequent isomerization of the endoperoxide product by PGE2 isomerase. PGE2 is produced by a variety of cells, including airway smooth muscle, epithelial cells, alveolar macrophages, and pulmonary endothelial cells (12, 23). In addition to many immunomodulatory effects, PGE2 also has effects on airway smooth muscle and nerves, which allow it to modulate airway caliber (4, 16, 22).

PGE2 mediates its action via G protein-coupled receptors to alter cyclic nucleotide levels in cells or stimulate phosphatidylinositol, thereby causing effects such as airway smooth muscle relaxation or uterine muscle contraction. Four types of cell surface receptors have been identified for which PGE2 is the cognate ligand. These EP receptors modulate a variety of effects. EP1 receptors binding PGE2 signal via G proteins to activate phospholipase C, generate phosphatidylinositol, mobilize calcium, and cause a chloride current (for review see Ref. 2). In contrast, EP2 and EP4 receptors characteristically relax smooth muscle by signaling through a Gs protein to increase intracellular cAMP levels. The EP3 receptor consists of a number of splice variants displaying various degrees of constitutive activity (6). EP3 signals through activation of a Gi protein to inhibit cAMP generation.

In vitro PGE2 causes relaxation of airway smooth muscle and also has effects on airway cholinergic nerve activity (8, 20). For example, PGE2 released from epithelial cells inhibits vagal cholinergic efferent transmission via a prejunctional mechanism (11). Human subjects inhaling PGE2 may show airway constriction or bronchodilation, which may in part be dependent on the timing of measurements after PGE2 administration (10, 19, 21). The relaxation of airway smooth muscle is thought to result from activation of EP2 and, possibly, EP4 receptors. To examine the role of EP2 receptors in mediating the many effects of PGE2, we used homologous recombination to construct a mouse lacking the EP2 receptor (7). We found that bronchodilation of mouse airways by PGE2 is dependent on signaling through the EP2 receptor.

METHODS

The disruption of the EP2 receptor in the mouse is described elsewhere (7). Briefly, the mice appear to have a normal phenotype but are less fertile, develop a greater degree of hypertension when fed a high-salt diet, and do not respond to infused PGE2 with hypotension. F2 C57/B6X129 mice homozygous for the targeted deletion of the EP2 receptor gene and littermate controls underwent measurements of a dimensionless index thought to represent airflow obstruction (enhanced pause; Penh) by barometric plethysmography (Buxco Electronics, Troy, NY) (5). A nebulizer (Pari II, Pari Respirator Equipment, Richmond, VA) was used to expose mice to aerosols of various concentrations of agonists. Methacholine (Sigma Chemical, St. Louis, MO) dose-response curves were determined by measuring Penh in response to aerosols of methacholine at concentrations of 12.5, 25, and 50 mg/ml for 2 min at 2-min intervals. Dose-response curves were constructed after inhalation of placebo, the mscarine antagonist ipratropium bromide (200 µg/ml; Roxane Laboratories, Columbus, OH), or PGE2 (100 µg/ml; Cayman Chemical, Ann Arbor, MI). To investigate the action of PGE2 on constricted airways, we produced airflow obstruction in mice with aerosols of methacholine (100 mg/ml for 4 min) followed by 2 min of PGE2 (100 µg/ml) or placebo (10% ethanol). The response over time in Penh was determined immediately after the 2-min aerosol. The effect of isoproterenol (1 mg/ml; Sigma Chemical), a nonspecific β-adrenergic agonist, was also determined.

To confirm the effects of PGE2 on airway mechanics, we measured lung resistance (RL) in anesthetized, tracheo-
mized, ventilated animals, as previously described (9, 15). Aerosols of saline, methacholine, or PGE2 were delivered to the inspiratory port of a Harvard ventilator. Increases in Rl were measured at baseline, immediately after cessation of a 2-min methacholine aerosol (100 mg/ml), and during aerosols of PGE2.

Statistics. Comparisons within groups were done using a paired t-test. Comparisons between groups of animals were done using an unpaired t-test. When the data were found to be nonnormal in distribution, a corresponding nonparametric t-test was applied. Significance was accepted at P < 0.05. Values are means ± SE.

RESULTS

There was no significant difference between the baseline measurements of Penh in wild-type and EP2(−/−) mice. Inhalation of PGE2 (100 µg/ml for 2 min) significantly increased Penh in wild-type and EP2(−/−) mice (Fig. 1). Serially increasing doses of aerosolized methacholine caused a dose-dependent increase in Penh in wild-type and knockout mice (Fig. 2). Although the EP2(−/−) mice had greater Penh measurements after methacholine than did the wild-type mice, this was not true of the larger group of mice studied after inhalation of 100 mg/ml of methacholine (see below). Prior inhalation of PGE2 (100 µg/ml for 2 min) significantly reduced the response to inhaled methacholine in the wild-type, but not the knockout, mice (Fig. 2). The muscarinic antagonist ipratropium bromide prevented methacholine-induced changes in Penh in both groups of animals (Fig. 3).

Methacholine aerosols (100 mg/ml for 4 min) induced transient increases in Penh in wild-type and EP2(−/−) mice that were not statistically different. Compared with placebo at the same time point, PGE2 (100 µg/ml for 2 min) reduced the airflow obstruction induced in wild-type mice by methacholine aerosols (Fig. 4). In marked contrast, inhalation of PGE2 failed to reduce methacholine Penh and was instead associated with a significant increase in this value in the EP2(−/−) mice.

The nonspecific β-agonist isoproterenol reduced the methacholine changes in Penh in both groups of mice (Fig. 5).

In anesthetized and mechanically ventilated animals, aerosols of methacholine were used to provoke increased Rl, and the effect of aerosols of PGE2 on the induced constriction was determined. The baseline Rl was similar in the two groups [1.28 ± 0.06 and 1.29 ± 0.05 ml·min·cmH2O−1 in wild-type and EP2(−/−) mice, respectively]. In wild-type mice, inhaled PGE2 caused a significant reduction in Rl; in contrast, inhaled PGE2 caused no significant change in Rl in the EP2(−/−) mice (Fig. 6).

There was no difference in the bronchoalveolar lavage cell counts or differentials between the two groups of animals (n = 4). Histological examination of the lungs of wild-type and EP2(−/−) mice did not reveal...
any evidence of differences in morphology between the two groups.

**DISCUSSION**

PGE₂ has complex effects on mammalian airway smooth muscle tone and on airflow. In humans, PGE₂ provokes cough and bronchoconstriction in some individuals, and in others it produces bronchodilation (10). PGE₂ is active on sensory nerve terminals, smooth muscle, mucous glands, and a variety of inflammatory cells. Part of the complex effects of PGE₂ may result from the variety of cells affected, but further complexity is introduced by the presence of a variety of receptor subtypes for PGE₂ that have different signaling characteristics. Thus the EP₁ receptor is thought to mediate constriction, and the EP₂ receptor is thought to mediate relaxation (13). Depending on the repertoire of receptor subtypes expressed in a given cell, binding of PGE₂ could have opposing effects (1).

Pharmacological probes for the various EP receptor subtypes have allowed considerable progress to be made in determining their function. The development of techniques for targeted gene disruption in the mouse and refinement of techniques for measurement of airway mechanics have allowed precisely defined experiments to be undertaken. We constructed a mouse lacking the EP₂ receptor subtype to explore the mechanism of PGE₂ signaling via this receptor. Because PGE₂ is an important autacoid in the lung, we sought to define the mechanism of PGE₂ action in wild-type and in littermate mice lacking the EP₂ receptor.

We used the technique of barometric plethysmography to measure airway responses. The technique measures box pressure difference between the animal chamber and a chamber vented to the atmosphere (5). From the box pressure, a variable, Penh or enhanced pause, is calculated. The value of Penh changes with alterations in breathing pattern, compression volume, and expiratory flow. Because several of these are linked to bronchoconstriction, the measurement of Penh often tracks with measures of airflow obstruction. We utilized this technique because it allows serial measurements in the same animals over time. We used conventional measures of RL to confirm the more important of our findings in anesthetized mechanically ventilated animals.

Inhaled PGE₂ increased Penh slightly in wild-type and EP₂(−/−) mice. This increase may have been related to stimulation of sensory nerve fibers provoking reflex bronchoconstriction. Neurally mediated bronchoconstriction has been demonstrated in an isolated...
canine tracheal segment when PGE$_2$ was delivered to the lower airway (17). In humans, aerosols of PGE$_2$ provoke cough and substernal discomfort and alterations in the sense of dyspnea, suggesting direct activation of sensory nerves (3). Because Penh is measured in conscious animals, airway sensory receptors responding to EP$_2$ stimulation could alter breathing pattern and Penh. The increase in Penh in wild-type mice and in mice lacking the EP$_2$ receptor suggests that if activation of sensory nerves is responsible, it must be mediated at least in part by another EP receptor such as EP$_1$ or EP$_3$.

Inhaled PGE$_2$ in wild-type mice consistently decreased measures of airflow obstruction, Penh or RL, when the animals had been preconstricted with methacholine. Similarly, PGE$_2$ aerosols prevented the increase in Penh caused by inhaled methacholine. In humans, PGE$_2$ has variable effects on histamine- or methacholine-induced bronchoconstriction (10, 14, 21). The difference could lie in the timing of measurements, difference in regulation of smooth muscle tone by nerves in humans and mice, or different complements of EP receptor subtypes expressed in human and mouse airway.

In marked contrast to the bronchodilating properties of PGE$_2$ in wild-type mice, EP$_2$(-/-) mice manifested bronchoconstriction or no change after PGE$_2$. This provides evidence that the EP$_2$ receptor is the principal receptor responsible for airway smooth muscle relaxation in the mouse. The response of both groups of mice to the β-adrenergic agent isoproterenol was similar, thereby documenting that the mice had similar capacities for smooth muscle relaxation. The baseline measurement of Penh was the same in both groups of mice, as was the response to inhaled methacholine. This would suggest that tonic modulation of airway tone via the EP$_2$ receptor in mice does not occur. We conclude that signaling via the EP$_2$ receptor in the mouse is responsible for the bronchodilatory effects of PGE$_2$.

The increase in Penh in preconstricted EP$_2$(-/-) mice given aerosols of PGE$_2$ might result from further bronchoconstriction resulting from unopposed signaling via the EP$_1$ or EP$_3$ receptor. In guinea pig, the EP$_1$ receptor appears to mediate bronchial smooth muscle contraction (13). However, when PGE$_2$ is given alone to mice, the increase in Penh was small. Thus there must be some interaction between constriction with methacholine and the effects of PGE$_2$, which differ in the EP$_2$(-/-) and wild-type mice. In the anesthetized, mechanically ventilated mouse, the increase after PGE$_2$ aerosols in mice preconstricted with methacholine was not present, suggesting that anesthesia was preventing a neural response to PGE$_2$ that was responsible for the increase in Penh in the awake EP$_2$(-/-) animals. Barbiturate anesthesia, as used in our experiments on mechanically ventilated mice, has the potential to suppress neural responses (18). We suggest that in the wild-type mice the smooth muscle-relaxing effects of signaling through the EP$_2$ receptor dominate, resulting in a drop in Penh and RL. In the EP$_2$(-/-) animals, a neurally mediated increase in Penh, caused by signaling via EP$_1$, EP$_3$, or EP$_4$ receptors unopposed by EP$_2$ receptor activation, results in an increase in Penh.

We conclude that the EP$_2$ receptor in murine airways transduces airway dilatation and can reverse muscarinic airway narrowing. When the EP$_2$ receptor is absent, resting airway mechanics are not affected, but in the conscious animal with airway narrowing induced by methacholine, the lack of EP$_2$ signaling results in further airway narrowing mediated by neural mechanisms.

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