Modulation of airway responses by prostaglandins in young and fully grown rabbits

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Larsen GL, Loader J, Fratelli C, Kang J-K, Dakhama A, Colasurdo GN. Modulation of airway responses by prostaglandins in young and fully grown rabbits. Am J Physiol Lung Cell Mol Physiol 293: L239–L244, 2007. First published May 4, 2007; doi:10.1152/ajplung.00413.2006.—Maturational changes have been noted in neurally mediated contractile and relaxant responses in airways from New Zealand White rabbits. In this study, we focused on prostaglandins with bronchoprotective properties as potential modulators of airway tone in maturing rabbits. Tracheal rings from 1-, 2-, and 13-wk-old rabbits were assessed for neurally mediated contractile and relaxant responses produced by electrical field stimulation (EFS) of nerves in the presence and absence of the prostaglandin inhibitor, indomethacin (Indo). We also measured EFS-induced release of prostaglandin E2 (PGE2) and the stable metabolite of prostacyclin, 6-keto-prostaglandin F1α (6-keto-PGF1α). In the presence of Indo, EFS produced significant increases in contractile responses in segments from 1- and 2-wk-old animals but not in segments from 13-wk-old rabbits. Tracheal rings from 1- and 2-wk-old animals precontracted with neurokinin A (NKA) relaxed 100% in response to EFS when Indo was not in the bath. In rings from 13-wk-old animals, relaxation was 40%. With Indo, relaxation was abolished in 1-wk-old animals and reduced to 30% in the 2- and 13-wk-old groups. Buffer released from tissues from 1- vs. 2- and 13-wk-old animals. Dose response curves to PGE2 using tissues precontracted to NKA showed significant increases in relaxant responses in 1- and 2-vs. 13-wk-old rabbits. In rabbit airways, this study demonstrates enhanced modulation of airway tone by PGE2 and greater release of the bronchoprotective prostaglandins PGE2 and prostacyclin early in life.

Keywords: airway development; bronchoprotection; prostacyclin; prostaglandin E2; relaxant responses of airways

The airways are abundantly supplied with nerve fibers that contain several neuropeptides that are important in neural control of airway function (22). The production and release of neuropeptides including those with opposing actions is thought to influence the patency of airways and their responsiveness as well as airway inflammatory responses (3, 6). For example, the peptide substance P (SP) is a well-known bronchoconstrictor of the nonadrenergic noncholinergic excitatory (NANCe) pathway in most species including humans (20). On the other hand, vasoactive intestinal peptide (VIP) is one of the many potential bronchodilators of the nonadrenergic noncholinergic inhibitory (NANCi) pathway (1, 14). The balance of bronchoconstrictive neuropeptides such as SP to bronchoprotective neuropeptides such as VIP may play a role in determining airway tone as well as the level of responsiveness to endogenous and exogenous stimuli. However, studies in humans (19) as well as mammalian models (5, 13, 26) suggest that these neural pathways may normally undergo significant postnatal developmental changes making neural control of airway caliber and responsiveness a dynamic process influenced in part by the stage of development as well as disease processes (22, 24, 25).

In addition to neuropeptides, contraction and relaxation of airways can be mediated and/or facilitated by other products generated within an airway. For example, eicosanoid mediators that include leukotrienes, prostaglandins, and thromboxanes are all bronchoactive. Among this group, prostaglandin E2 (PGE2) is a dominant cyclooxygenase product of airway epithelium and smooth muscle and is thought to be predominately bronchoprotective (31). Support for this latter statement rests in part on the observations that PGE2 inhibits exercise-induced bronchoconstriction (28) and allergen-induced early and late asthmatic responses (11, 32).

Despite a growing literature related to developmental changes in neural control of airways, little is known about normal age-related changes in the bronchoprotective effect of prostaglandins such as PGE2 and prostaglandin I2 (prostacyclin, PGI2). The purpose of this study was to expand our knowledge in this area by addressing the effects of these bronchoprotective substances within airways of 1-, 2-, and 13-wk-old (fully grown) rabbits. This is a mammalian species in which significant information is available on normal neural mechanisms of airway control as a function of age (5, 23, 26). For this work, we addressed the hypothesis that the amount of these bronchoprotective prostanoids generated within an airway may change as a function of normal development and maturation. Furthermore, we addressed the hypothesis that the bronchoprotective role of the predominant cyclooxygenase product (PGE2) may also vary with age.

Materials and Methods

Experimental animals. New Zealand White rabbits were bred locally in Denver, CO (altitude 5,280 ft) to produce litters for study. All procedures employed in this work were approved by the Animal Care and Use Committee at the National Jewish Medical and Research Center and conformed to National Institutes of Health guide-
Tissue preparation and equilibration. Segments of tracheal smooth muscle (TSM) were obtained for these studies using previously described techniques (12). Briefly, the animals were killed using xylazine (50 mg/kg) and ketamine (100 mg/kg). Segments of TSM that were 0.75–1 cm in length were obtained from normal rabbits at 1, 2, and 13 wk of age. When the experiment called for studies to be done in the presence and absence of indomethacin (Indo), two contiguous segments of airway were obtained from each rabbit to be used for the two experimental conditions (below). The tracheal segments were removed and placed in modified Krebs-Henseleit (KH) solution. The airway segments were cleaned of loose connective tissue and placed in 3-ml organ baths (Harvard Apparatus, Holliston, MA) containing modified KH solution aerated with a 95% O2, 5% CO2 gas mixture at a pH of 7.43 ± 0.03. The temperature of the baths was maintained at 37°C. To minimize any residual effects of the medications used for euthanasia, the solution in the bath was changed nine times before study of the tissue to obtain data for the study. The time between solution changes was 15 min each.

Assessment of contractile and relaxant responses in TSM to electrical field stimulation. Electrical stimuli were delivered by a Grass Instruments S44 stimulator connected to a Stimu-Splitter II (Med-Lab, Loveland, CO). Stimulation of nerves via electrical field stimulation (EFS) was applied transmurally across the tissues with parallel platinum electrodes (each 0.3 cm²). Contractile responses were assessed on contiguous tracheal segments in the presence and absence of Indo (final concentration 1 × 10⁻⁵ M; Sigma, St. Louis, MO), utilizing increasing frequencies from 0.5 to 30 Hz. Results were expressed in terms of the frequency of stimulation causing 50% of the maximal contractile response (ES₅₀). When assessing relaxation, experiments were performed in the presence of atropine (final concentration in the bath of 1 × 10⁻⁶ M; Aldrich, Milwaukee, WI) and propranolol (final bath concentration of 5 × 10⁻⁶ M; Sigma). These drug concentrations have been previously used by our laboratory (5, 9) and others (7) to study NANC responses in vitro. Neurokinin A (NKA; final concentration 5 × 10⁻⁶ M; Sigma) was used to induce TSM tone. This concentration of NKA produced ~50% of the maximal contractile response. EFS was applied at a stimulation frequency of 20 Hz. Changes in tension from the baseline NKA contractile response were recorded and expressed as percent relaxation (mean ± SE). Studies involving relaxation were also performed in the presence and absence of Indo on two adjacent tracheal rings.

Stimulation of prostanooid release and assays for PGE₂ and 6-keto-prostaglandin F₁₅o. Tracheal rings were equilibrated in 1 ml of KH buffer and contracted with NKA (5 × 10⁻⁶ M) in the presence of atropine (1 × 10⁻⁶ M) and propranolol (5 × 10⁻⁶ M). Alternating tissues of adjacent tracheal rings also had Indo (1 × 10⁻⁵ M). Electrical stimuli were delivered by a Grass Instruments S44 stimulator connected to a Stimu-Splitter II (Med-Lab). Stimulation of nerves via EFS was applied transmurally across the tissues by means of parallel platinum electrodes (each 0.3 cm²). This stimulation was applied to tissues for 5 min at a frequency of 10 Hz. Bath buffer was immediately removed, filtered through 0.22-μm syringe filters into microcentrifuge tubes, and frozen at −70°C. Tubes were thawed and assayed immediately by ELISA using enzyme immunosassay kits supplied by Cayman Chemical (Ann Arbor, MI). A mouse monoclonal antibody for PGE₂ and a rabbit polyclonal antibody for the stable metabolite of prostacyclin, 6-keto-prostaglandin F₁₅o (6-keto-PGF₁₅o), were utilized. Assays were performed according to the instructions provided with each kit.

Dose response curves for PGE₂. Tracheal tissue was obtained from normal New Zealand White rabbits as outlined above. Segments of trachea were equilibrated in KH solution at an optimal resting tension of 1.5 g. Tissues were challenged twice with 120 mM KCl and then washed until they returned to resting tension values. Dose response curves to PGE₂ were performed in the presence of atropine at a final concentration of 1 × 10⁻⁶ M and propranolol at a final concentration of 5 × 10⁻⁶ M. After obtaining a plateau response with NKA at a final concentration of 5 × 10⁻⁶ M, PGE₂ (Sigma) was added in whole log doses ranging from 10⁻⁶ to 10⁻⁵ M. Changes in tension from the baseline NKA contractile response were recorded and expressed as percent relaxation (mean ± SE). The concentration of PGE₂ that produced 50% of the maximal relaxant response (EC₅₀) was calculated.

Statistical analyses. Results are expressed as means ± SE, where n equals number of experimental observations (i.e., the number of TSM segments and animals studied). Under any experimental condition, no more than one segment was obtained from an individual rabbit. The studies shown in Figs. 1–4 (below) were performed on separate groups of rabbits. A one-way ANOVA was used in data analysis with the Tukey-Kramer honestly significant difference test of multiple comparisons applied to the analysis. P < 0.05 was considered significant.

RESULTS

Values for the frequency (Hz) of EFS that produced 50% of the maximal contractile response (ES₅₀) to this neural stimulus are shown in Fig. 1 for tracheal rings from 1-, 2-, and 13-wk-old rabbits. For each age group, experiments were conducted either in the presence (closed bars) or absence (open bars) of Indo. Significant increases in the response to EFS were found in rings from the two youngest groups when Indo was present in the tissue baths (P < 0.05). This is indicated by decreases in values of ES₅₀. For example, the ES₅₀ value in segments from 1-wk-old rabbits was 4.5 ± 0.5 Hz when Indo was not present for the experiments and fell to 2.9 ± 0.3 Hz when Indo was present. Similarly, the value in tissue from 2-wk-old rabbits fell from 5.0 ± 0.8 to 2.7 ± 0.4 without and with Indo, respectively. Conversely, the presence or absence of Indo did not alter the response to EFS in fully grown rabbits. These observations suggest generation of prostanooids can modulate (decrease) cholinergic contractile responses in younger but not fully grown animals.
The percent relaxation in response to EFS is shown in Fig. 2 for tracheal rings from 1-, 2-, and 13-wk-old rabbits. For each group, ~50% of a maximal contraction was first produced by NKA. In assessing each age group, experiments were conducted either in the presence (closed bars) or absence (open bars) of Indo. In the presence of Indo, a relaxant response was not seen in rings from 1-wk-old rabbits but was present at both 2 and 13 wk of age. In the absence of Indo, complete (100%) relaxation of the NKA-induced contractions was achieved in rings from 1-wk-old rabbits with 95.8 ± 2.3% relaxation noted at 2 wk of age. The differences in relaxation as a function of the presence or absence of Indo were significant \((P < 0.05)\) in the tissues from the two younger age groups. Conversely, the relaxant response was not significantly altered in the absence of Indo in rings from the oldest group of rabbits.

Values for PGE2 and 6-keto-PGF1α (pg/mg of tissue per minute of stimulation) found in tissue baths in response to EFS are shown in Fig. 3 for tracheal rings obtained from normal rabbits at the three postnatal ages. Experiments were conducted either with or without Indo in the baths. The pattern was similar for both prostanoids. The amounts present were highest in rings from the youngest group and decreased significantly \((P < 0.05)\) with age when Indo was not present in the baths. The values for PGE2 were 23.4 ± 2.9, 3.2 ± 1.5, and 1.3 ± 0.6 pg/mg of tissue per minute of stimulation in segments from 1-, 2-, and 13-wk-old rabbits. Values for the stable metabolite of prostacyclin were 8.4 ± 2.4, 3.0 ± 0.8, and 0.8 ± 0.4 pg/mg of tissue per minute of stimulation in segments from 1-, 2-, and 13-wk-old rabbits. Thus generation of each prostanoid decreased more than 10-fold from the youngest to oldest group. In the presence of Indo, significantly less PGE2 and 6-keto-PGF1α were found in the baths at each age \((P < 0.05)\).

Relaxant responses to PGE2 were also defined for tracheal rings from 1-, 2-, and 13-wk-old rabbits (Fig. 4). The numbers of rings from each group were six, four, and four, respectively. For these studies, contraction was first produced by NKA. Relaxant responses were greater in rings from the two youngest groups. For example, complete (100%) relaxation was noted at 10^{-7} M PGE2 for both younger groups whereas the value for the rings from 13-wk-old rabbits was 56.8 ± 9.8%. The EC_{50} values of the two youngest groups were also significantly different from the oldest group \((P < 0.05)\). The values were 8.1 ± 0.5 × 10^{-9}, 6.7 ± 0.2 × 10^{-9}, and 7.4 ± 0.7 × 10^{-8} M for rings from 1-, 2-, and 13-wk-old rabbits, respectively. Thus there was approximately a log difference in the response of rings from each group.
of tracheal segments to this prostanoid when comparing tissues from the youngest and oldest groups.

**DISCUSSION**

If we are to understand the normal pathways that affect control of airway function including the level of responsiveness, defining the interplay between bronchoconstrictive and bronchoprotective products as a function of postnatal age is important. These bronchoactive products may originate from nerves and be part of the pathways that mediate neural control or may be produced from nonneural elements within airways. For both neural and nonneural pathways, there is evidence that they change as part of normal development. In terms of the former, age-related differences in innervation of airways in humans has been described (19). Clinical observations such as this are limited given the difficulty of obtaining specimens for study. However, they do suggest that the process of supplying neural control to airways is dynamic and changes with age. In models, neurotrophic factors that are important in neural development as well as neuropeptides that modulate effects on airways may change as a function of normal development. In this respect, we previously addressed normal temporal changes within rabbit airways in terms of NGF and SP (26) by defining the quantity of this neurotrophic factor and this neuropeptide within airways. In that work, both NGF and SP were elevated in homogenates of TSM from 2-wk-old rabbits compared with homogenates from 13-wk-old animals. Levels of VIP in tissue homogenates were not assessed in that work. However, Geppetti et al. (13) found age-related differences in VIP concentrations in rats with ~60% reduction as the animals aged. In subsequent work from our laboratory (23), we found that release of NGF as well as SP and VIP in response to EFS decreased as a function of postnatal age in normal rabbits.

This current study addressed the effects of normal maturation on a nonneural pathway of bronchoprotection within airways. This work shows enhanced modulation of airway tone by PGE2 and greater generation of potentially bronchoprotective prostanoids early in life. This is seen in a species in which the NANCi response is not normally present at the earliest time point examined (5). The magnitude of relaxation associated with generation of prostanoids is greater than that achieved with classical NANCi responses, suggesting these compounds are potent bronchoprotective agents. Thus generation of prostanoids such as PGE2 and PGI2 relax airways early in life in this species before a fully mature NANCi pathway is present. However, it is important to note that this effect of PGE2 may be very dependent on the age of the animal as suggested in Fig. 4 where approximately a log difference in the EC50 for relaxation is found between segments of trachea from younger (1- and 2-wk-old rabbits) compared with fully grown 13-wk-old animals. The potential reasons for this age-related difference in response include the possibility that receptors for this prostanoid are altered as a function of age. PGE2 can activate four high-affinity seven-transmembrane G-coupled protein receptors referred to as EP1 to EP4 (29). Tilley and colleagues (36) reported that airway constriction in mice in response to PGE2 was mediated indirectly through EP1 and EP3 receptors via neural pathways whereas PGE2-induced bronchodilation resulted from direct activation of EP2 receptors on airway smooth muscle. In other studies using murine models to assess the ability of PGE2 to alter airway function, genetic manipulations that led to elevation of PGE2 levels in the lung attenuated airway responsiveness to cholineric stimuli (18) with the protective action mediated largely through the EP3 receptor. In an ovalbumin-induced model of allergen-induced bronchoconstriction in guinea pigs, Tanaka et al. (35) reported that PGE2 acts via EP2 and EP4 receptors to inhibit allergen-induced airway obstruction. In humans, relaxation of isolated bronchial preparations was induced by EP2 but not EP4 receptors (30). Thus studies from models and humans have found that PGE2 has direct bronchoprotective properties mediated by EP2 receptors. The possibility that this or other receptors for this prostanoid may change as a function of age or disease state in man is not well defined. However, there are observations that suggest that alterations in EP2 receptor numbers may be part of a disease process. Ying et al. (40) found an increased percentage of macrophages in induced sputum expressing EP2 and EP4 in patients with asthma compared with control subjects. Burgess and colleagues (2) found an increased number of EP2 receptors on airway smooth muscle from asthmatics compared with normal subjects. Ying et al. (39) reported that aspirin-sensitive rhinosinusitis is associated with increased expression of EP2 receptors in the nasal epithelium but reduced EP2 receptor expression on nasal mucosal inflammatory cells. Thus precedents for change in the numbers of these receptors with pathological processes exist. The numbers of receptors that are normally found as a function of age as well as their distribution within airways remains to be addressed in future work.

The bronchoprotective properties of PGE2 are complex and involve not only acute improvements in airway function (21, 32), but also effects on airway caliber that are likely mediated through the anti-inflammatory properties of the prostanoid (11,
In the model used for this work, segments of normal airway smooth muscle were employed making the observations most relevant to an airway not subjected to stimuli that lead to airway inflammation. The potential changes in prostanooid production and the direct response of the airway to PGE2 when the airway has been subjected to stimuli that lead to inflammation (allergen sensitization/challenge, infection, etc.) are currently undefined in this and other models of airway development.

Comment is needed regarding the generation of prostanooids via EFS. Past work from three separate laboratories all found that tetrodotoxin does not block release of various prostaglandins from segments of ferret (37), guinea pig (10), and canine trachea (27). In all studies, PGE2 was released from the preparations of tracheal segments independent of nerve function sensitive to this agent. The mechanism by which this occurs was not defined in any of these manuscripts or in the current study. We do know that contraction of segments of rabbit trachea with cholinergic agonists and NKA do not lead to release of the two prostanooids that were the focus of this investigation (J. Loader and G. L. Larsen, unpublished observations). Thus the generation of PGE2 (and possibly prostacyclin) may be due to activation of tetrodotoxin-resistant nerves (37) with secondary generation of this prostanooid or may be related to a nonneural effect of EFS on other cell types including mast cells and neuroendocrine cells (27).

The effects of bronchoactive products in various age groups may be determined by amount released as well as the location of release within an airway. In addition, within any one location, the effects of these products may be determined by the balance of neural and nonneural products. The concept of considering the balance of bronchoactive products in terms of the overall effect is supported by observations from models as well as clinical investigations. As an example of the former, we reported that SP increased release of acetylcholine from neural terminals in a receptor-mediated fashion whereas VIP prevented this increase without directly influencing acetylcholine release (6). Thus, if only SP is present around the nerve, upregulation of cholinergic responses should occur due to greater neural release of acetylcholine whereas SP in the presence of VIP may not have this potentiating effect on neural transmission. In terms of the bronchoprotective actions of prostanooids, PGE2 can suppress acetylcholine release from parasympathetic nerves (34). In human airways, Ellis and Conanan (8) found PGE2 inhibited cholinergic responses. In terms of potential clinical relevance based on study of humans, Cardell and associates (3) found a relationship between acute exacerbations of asthma in adults and low plasma concentration of VIP together with elevated levels of other neuropeptides including SP. All these observations suggest that the balance of bronchoactive products with opposing actions may be important in determining manifestations of disease. Additional clinical studies together with work with models are needed to expand our insight into the complexity of control of airway function.

In summary, this study demonstrates enhanced modulation of airway tone by PGE2 and greater release of the bronchoprotective prostaglandins PGE2 and prostacyclin early in life. The degree of bronchoprotection provided by this nonneural pathway is greater than that normally seen in terms of the NANCi response in this species. In addition, this pathway is active early in life before a functional NANCi response is present in this species. The possibility that this as well as other pathways complement neurally mediated bronchoprotection as a function of both normal development and in the face of disease merits additional study.

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REFERENCES