Hyperoxia impairs airway relaxation in immature rats via a cAMP-mediated mechanism

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Mhanna, Maroun J., Musa A. Haxhiu, Marwan A. Jaber, Ronald W. Walenga, Chang-Ho Chang, Shijian Liu, and Richard J. Martin. Hyperoxia impairs airway relaxation in immature rats via a cAMP-mediated mechanism. J Appl Physiol 96: 1854–1860, 2004. First published February 6, 2004; 10.1152/japplphysiol.01178.2002—Hyperoxic exposure enhances airway reactivity in newborn animals, possibly due to altered relaxation. We sought to define the role of prostaglandin- and nitric oxide-mediated mechanisms in impaired airway relaxation induced by hyperoxic stress. We exposed 7-day-old rat pups to either room air or hyperoxia (>95% O2) for 7 days to assess airway relaxation and cAMP and cGMP production after electrical field stimulation (EFS). EFS-induced relaxation of precontracted trachea was diminished in hyperoxic vs. normoxic animals (P < 0.05). Indomethacin (a cyclooxygenase inhibitor) reduced EFS-induced airway relaxation in trachea from normoxic (P < 0.05), but not hyperoxic, rat pups; however, in the presence of Nω-nitro-l-arginine methyl ester (a nitric oxide synthase inhibitor) EFS-induced airway relaxation was similarly decreased in trachea from both normoxic and hyperoxic animals. After EFS, the increase from baseline in the production of cAMP was significantly higher in trachea from normoxic than hyperoxic rat pups, and this was accompanied by greater prostaglandin E2 release only in the normoxic group. cGMP production after EFS stimulation did not differ between normoxic and hyperoxic groups. We conclude that hyperoxia impairs airway relaxation in immature animals via a mechanism primarily involving the prostaglandin-cAMP signaling pathway with an impairment of prostaglandin E2 release and cAMP accumulation.

CHRONIC NEONATAL LUNG DISEASE, also known as bronchopulmonary dysplasia (BPD), is a major complication of prematurity that affects a high proportion of very-low-birth-weight infants (4). High inspired O2 delivered to an immature respiratory and antioxidant (25) system appears to be an ingredient for development of BPD, although this disorder may be seen in very premature infants in whom the initial exposure to increased O2 may only be of several days duration. Nonetheless, the functional and pathological effects of hyperoxic exposure in neonatal mice suggest a lesion very similar to BPD (39). Although the lungs of premature infants may suffer from arrested alveolarization, interstitial edema, decreased vascular branching, dysplastic airway epithelium, and inflammation (26, 27), increased airway responsiveness and lung resistance are typically an early and prominent manifestation of developing BPD (4).

Hyperoxic exposure over 8 days has been demonstrated to increase cholinergic responsiveness in newborn guinea pigs (33, 38) and rat pups, associated with epithelial and smooth muscle layer thickening (20). However, several studies, including those from our laboratory, have shown that increased cholinergic responsiveness of airways from hyperoxia-exposed immature rats cannot be attributed primarily to an increase in smooth muscle mass (3, 21, 36). Because maintenance of a homeostatic balance between airway smooth muscle relaxant and contractile responses is critical for regulation of airway patency during postnatal maturation, there has been considerable interest in the role of nonadrenergic noncholinergic inhibitory neural mechanisms that mediate airway relaxant responses (5). Substances such as nitric oxide (NO) and prostaglandin E2 (PGE2), which are mainly released from epithelial cells or possibly smooth muscle cells in the airway or lung parenchyma, are potent airway smooth muscle relaxants (17, 34). In allergen-challenged rabbits and respiratory syncytial virus-infected rats and ferrets, there is evidence for impairment of such nonadrenergic inhibitory mechanisms, as elicited by electrical field stimulation (EFS) (9–11, 15). However, the neurotransmitter-mediated mechanisms underlying changes in the airway smooth muscle relaxant responses of healthy and diseased animals have not been clearly characterized.

In this study, we sought to investigate the effect of hyperoxic exposure on airway smooth muscle relaxation in immature rat pups and determine whether there is an impairment of relaxation similar to that observed in allergen-challenged or respiratory syncytial virus-infected immature animals. We hypothesized that hyperoxic exposure decreases the ability to mount a compensatory relaxant response to bronchoconstriction in early life and that decreased production of relaxant prostaglandins and NO may be responsible for this impairment of airway relaxation. To test our hypothesis, we used EFS as a means to induce airway relaxation of precontracted trachea in vitro, because EFS-induced airway relaxation is mediated partly via the release of PGE2 and nonadrenergic noncholinergic substances such as NO (6, 37).

MATERIALS AND METHODS

Animal preparation and physiological studies. Seven-day-old Sprague-Dawley rat pups were exposed to hyperoxic or normoxic conditions for 7 days. Rat pups from several litters were weighed, mixed, and assigned to hyperoxic and to normoxic groups. Hyperoxic groups were placed in a Plexiglas chamber (38 liters) and exposed to increased O2 may only be of several days duration. Nonetheless, the functional and pathological effects of hyperoxic exposure in neonatal mice suggest a lesion very similar to BPD (39). Although the lungs of premature infants may suffer from arrested alveolarization, interstitial edema, decreased vascular branching, dysplastic airway epithelium, and inflammation (26, 27), increased airway responsiveness and lung resistance are typically an early and prominent manifestation of developing BPD (4).

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continuous flow of O₂ (12 l/min). O₂ concentration was maintained at >95% in the chamber and monitored four times daily with a Miniox III (Echidyne) O₂ monitor. There was no addition of CO₂ to the chamber under normoxic or hyperoxic conditions. Furthermore, no retention of CO₂ occurred under either condition. The pups assigned to normoxia were kept in an open cage. To ensure pup and maternal survival, mothers were switched on alternate days between normoxic and hyperoxic conditions. After 7 days of exposure, animals were killed by exposure to CO₂, and their trachea removed, as our laboratory has previously described (3). A cylindrical airway segment of 3-mm length was isolated from the midtrachea of each animal and placed in a modified Krebs Henseleit (KH) solution. Tracheal cylinders were suspended between a sturdy glass rod and a force displacement transducer and were continuously oxygenated with a gas mixture containing 95% O₂-5% CO₂. This exposure to hyperoxic conditions occurred for both normoxic- and hyperoxic-exposed tissues to ensure their optimal viability. Therefore, observed differences between groups could not be explained by hyperoxic exposure during tissue incubations. Generated forces were continuously monitored and recorded as our laboratory has previously described (3).

A cumulative concentration-response curve to bethanecol and the resultant concentration of bethanecol that elicited 50–75% of maximal response (ED₅₀–₇₅) were obtained for trachea from both normoxic (n = 8) and hyperoxic (n = 8) pups. Bethanecol at 10⁻⁷ M was found to be the optimal concentration necessary to elicit 50–75% of maximal response in both normoxic and hyperoxic animals. The airway cylinders were then washed, equilibrated, and preconstricted with bethanecol at their ED₅₀–₇₅. EFS, in which direct current was employed, was then applied for 10 s to each preconstricted cylinder at 4, 5, 6, 7, and 8 V separated by 4-min intervals. The percent relaxation from the preconstricted state was calculated for each cylinder as previously described (3, 30).

Tracheae from subsequent groups of normoxic (n = 8) and hyperoxic (n = 8) animals were incubated with the cyclooxygenase inhibitor indomethacin at 10⁻⁶ M for 30 min, a concentration previously described by our laboratory and others (9, 30, 37). The chosen concentration of indomethacin has previously been shown to inhibit prostaglandin-induced relaxation by EFS (9). Tracheal cylinders were then preconstricted with bethanecol (10⁻⁵ M) and exposed to EFS at the above voltages (from 4 to 8 V). In addition, normoxic (n = 8) and hyperoxic animals (n = 8), preconstricted tracheal segments were exposed to a NO synthase (NOS) inhibitor, N⁶-o-nitro-L-arginine methyl ester (L-NAME), at 10⁻⁴ M for 30 min before bethanecol (30) followed by EFS stimulation as described above. As in the earlier subgroup, cylinders only exposed to bethanecol followed by EFS served as controls. L-NAME at 10⁻⁴ M was employed because this concentration was 10-fold higher than the concentration of NONOate (NO donor) that induced 100% relaxation of precontracted trachea (based on dose-response curve of tracheal relaxation to NONOate administration).

To determine whether PGE₂-induced airway relaxation is impaired in hyperoxic vs. normoxic animals, precontracted airway segments from normoxic and hyperoxic animals were exposed to two concentrations of PGE₂ (10⁻⁶ and 10⁻⁸ M), well characterized for their effects in our previous studies (30).

**cAMP and cGMP measurements.** An additional series of normoxic (n = 32) and hyperoxic (n = 32) animals were killed, and cylindrical airway segments were isolated from the midtrachea of each animal and placed in a modified KH solution. As for our physiological studies, tracheae were incubated with bethanecol at 10⁻⁷ M for 3 min to elicit a 50–75% maximal contraction and were immediately frozen in liquid N₂ and stored at −80°C to serve as controls. Tracheae from other animals were additionally exposed to EFS at 5 V for 10 s and then frozen in liquid N₂ at 30, 60, and 180 s after completion of EFS. After homogenization of the tracheal tissues, cAMP and cGMP generation was determined by using commercially available radioimmunoassays, with ¹²⁵I-labeled cAMP and ¹²⁵I-labeled cGMP as tracers (Amersham Pharmacia Biotech). Measurements of cAMP and cGMP production were performed in the absence of phosphodiesterase inhibition. The tissues’ protein concentration was assayed by using the Lowry method.

**Measurements of PGE₂ production.** An additional series of normoxic (n = 9) and hyperoxic (n = 9) animals were killed, and cylindrical airway segments were isolated from the midtrachea of each animal and placed in a modified KH solution. As for our physiological studies, tracheae were incubated with bethanecol at 10⁻⁷ M for 3 min to elicit a 50–75% maximal contraction, followed by EFS at 5 V for 10 s. Fluids were collected from the organ bath at 0 s (before EFS) and at 60 s and 180 s after EFS. PGE₂ content of tissue supernatant (after concentration under vacuum) was assessed by direct enzyme immunoassay using specific anti-prostaglandin antisera and acetylcholinesterase-coupler tracer (Cayman Chemical, Ann Arbor, MI), by using a modification of a previously described technique (31). Statistical analysis. Comparisons among the various experimental groups were made by using an ANOVA. One-way ANOVA and post hoc comparisons (Newman-Keuls’s test) were used to compare cAMP and cGMP amounts, respectively, in hyperoxic and normoxic rat pups over time. ANOVA was also used to assess PGE₂ production with unpaired t-test employed for baseline comparisons. Unpaired t-test was also used to analyze the response to PGE₂ in tracheae from normoxic and hyperoxic rats. All data are expressed as means ± SE.

**RESULTS**

**Tracheal smooth muscle relaxant responses.** EFS-induced relaxation of preconstricted trachea in the hyperoxic group was decreased compared with the normoxic group (Fig. 1). The percent relaxation varied from 19.1 ± 2.8% (at 4 V) to 54.8 ± 4.2% (at 8 V) in the tracheae from normoxic animals (n = 8) and from 16.1 ± 3.0% (at 4 V) to 41.8 ± 4.6% (at 8 V) in the tracheae from hyperoxic animals (n = 8).

To determine whether prostaglandins are involved in mediating relaxation induced by EFS, trachea from normoxic and

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**Fig. 1.** Electrical field stimulation (EFS) induced less relaxation of precontracted trachea in the hyperoxic group (n = 8) vs. the normoxic group (n = 8; 2-way ANOVA, P = 0.03).
hyperoxic animals were incubated with indomethacin before EFS. In the presence of indomethacin, EFS-induced airway relaxation was significantly decreased in tracheae from normoxic animals (Fig. 2A), but it remained unchanged in tracheae from hyperoxic animals (Fig. 2B). To determine whether NO is involved in the mechanism of relaxation induced by EFS, additional tracheae from normoxic and hyperoxic animals were incubated with L-NAME before EFS. In the presence of L-NAME, EFS-induced airway relaxation was similarly decreased in tracheae from both normoxic (Fig. 3A) and hyperoxic rats (Fig. 3B).

To determine whether PGE$_2$-induced airway relaxation is impaired, precontracted airway segments from hyperoxic and normoxic rats were exposed to two different concentrations of PGE$_2$. The percent relaxation of precontracted trachea to PGE$_2$ (at $10^{-8}$ M) was similar between tracheae from normoxic and hyperoxic animals [37.2 ± 8.24% ($n = 10$) vs. 31.7 ± 8.1% ($n = 10$), respectively]. The percent relaxation of precontracted trachea to PGE$_2$ at a higher concentration ($10^{-6}$ M) was also similar between tracheae from hyperoxic and normoxic animals [44.4 ± 10.4% ($n = 7$) vs. 48.4 ± 5.9% ($n = 7$), respectively].

**cAMP measurements.** After incubation with bethanecol, baseline cAMP was 2.4 ± 0.1 fmol/μg of protein in the tracheae from normoxic animals ($n = 8$) and 4.2 ± 0.2 fmol/μg of protein in the tracheae from hyperoxic animals ($n = 8$; $P < 0.01$). At subsequent time points after EFS, absolute cAMP values did not differ between tracheae from the two groups. There was a highly significant increase in cAMP in the normoxic group, which was greatest in the tissues sampled 60 s after EFS. In contrast, the response of cAMP production in the hyperoxic group was modest and did not reach statistical significance (Table 1). Post hoc analysis showed a significant difference between cAMP levels at baseline and 30 and 60 s after EFS in tracheae from normoxic rats ($P < 0.05$). In tracheae from hyperoxic rats, post hoc analysis showed a significant difference only between cAMP levels at baseline and 60 s ($P < 0.05$). When expressed as a percent change in cAMP production after EFS over time, the response of the normoxic group was significantly greater than the response of the hypoxic group (Fig. 4).

**cGMP measurements.** After incubation with bethanecol, baseline cGMP was 79.8 ± 22.3 fmol/μg of protein in the tracheae from normoxic animals ($n = 8$) and 89.3 ± 26.4 fmol/μg protein in the tracheae from hyperoxic animals ($n = 8$). As shown in Table 2, there was a variable response of
cGMP to EFS in the normoxic and hyperoxic groups, which was marginally significant in tracheae from the hyperoxic animals. Post hoc analysis showed a significant difference between cGMP baseline level and 180-s level in tracheae from normoxic rats ($P < 0.05$). Post hoc analysis also showed a significant difference between cGMP levels at 30 vs. 180 s in tracheae from hyperoxic rats ($P < 0.05$). When expressed as a percent change in cGMP production after EFS stimulation over time, the response of the normoxic group was not different from the hyperoxic group (Fig. 5).

**PGE$_2$ measurements.** After incubation with bethanecol, baseline PGE$_2$ was higher in tracheae from hyperoxic (130.7 ± 3.0 pg/ml) vs. normoxic (117.9 ± 4.3 pg/ml supernatant) animals ($P = 0.02$). In response to EFS, there was a significant increase in PGE$_2$ concentration between baseline and 180 s in the tracheae from normoxic animals, whereas there was no significant difference in the concentration of PGE$_2$ between 0 and 180 s in the tracheae from hyperoxic animals (Table 3).

**DISCUSSION**

Several authors have shown that hyperoxic exposure enhances airway reactivity in newborn animals (3, 13, 33, 38), but the effect of hyperoxia on airway relaxation has not been extensively studied. We have shown in our study that airway relaxation is impaired in a hyperoxia-exposed immature animal model and that this impairment is associated with diminished release of PGE$_2$ and production of cAMP compared with normoxic animals. Our results suggest that hyperoxic exposure decreases the ability to mount a compensatory relaxant response when exposed to bronchoconstrictor stimuli in early life and that impairment in PGE$_2$- and cAMP-mediated signaling mechanisms may be responsible. This may be analogous to previous reports of impaired EFS-induced tracheal relaxation observed in allergen-challenged rabbits and respiratory syncytial virus-infected rats and ferrets (9–11).

EFS induces airway relaxation in preconstricted airways partly via release of NO and prostaglandins (6, 37). Our laboratory and others have previously shown that substance P (SP), a tachykinin neuropeptide, induces relaxation of preconstricted airways via the release of endogenous prostaglandin in the presence of an intact airway epithelium (14, 30, 37). Devillier et al. (14) have shown that SP-induced airway relaxation is mediated through the activation of neurokinin NK$_1$ receptors on tracheal epithelium, leading to the release of PGE$_2$ and smooth muscle relaxation. The relaxant effect of SP and generation of PGE$_2$ were significantly decreased after tracheal epithelial damage or after incubation of epithelium-intact trachea with indomethacin. In our present study, indomethacin impaired EFS-induced airway relaxation in tracheae from normoxic, but not hyperoxic, animals; EFS induced less generation of PGE$_2$ and cAMP after hyperoxic exposure. Our findings are consistent with previous observations that hyperoxia decreases cyclooxygenase activity and PGE$_2$ production in endothelial (23) and tracheal epithelial cells (12). Hyperoxia has also been shown to inhibit prostacyclin production and cyclooxygenase activity in human umbilical arteries (35, 41). Therefore, depleted production of relaxant prostaglandins such as PGE$_2$ or prostacyclin from hyperoxia-exposed airway epithelium may contribute to enhanced contractile responses under these conditions.

Previous studies performed in piglet airways under in vitro conditions have demonstrated that release of endogenous NO opposes cholinergically induced contraction of tracheal smooth muscle in the presence of an intact epithelial layer (24). This is consistent with our present study in rat pups in which we have shown that EFS-induced relaxation of tracheal smooth muscle was accompanied by an increase in cGMP and blocked by the NOS inhibitor L-NAME. These observations did not differ between tracheae from normoxic and hyperoxic rat pups, leading us to speculate that hyperoxia does not affect primarily NO- and cGMP-mediated relaxant responses of tracheal smooth muscle. It is possible that our experimental design did not show a difference between tracheae from normoxic and hyperoxic rats because we used bethanecol to induce tracheal preconstriction. The use of bethanecol, a muscarinic agonist that is known to induce cGMP release in airways (8), might have increased the amount of cGMP production preventing a small, but significant, difference in cGMP concentrations to be detected after EFS stimulation in tracheae from normoxic vs. hyperoxic animals. The considerable variability in relaxant responses to EFS before and after NOS blockade may have

![Figure 4](https://www.jap.org/)

**Fig. 4.** The percent change in cAMP at specific times, after EFS stimulation, was higher in normoxic vs. hyperoxic animals. Each data point represents a mean ± SE derived from tracheae of 8 different animals (2-way ANOVA, $P < 0.001$).
under in vivo conditions that NOS blockade with L-NAME in animals by prior blockade of NOS. These observations mirror significantly potentiated in normoxic conditions. Iben et al. also showed previously described a potential interaction between NO and prostaglandins in SP-induced airway relaxation (6). The increased airway relaxation in response to pharmacological stimulation with β-adrenergic substances (reported by Fayon et al.) might represent a compensatory mechanism to the physiological impairment of airway relaxation seen in our study in response to endogenous stimulation of nonadrenergic noncholinergic and prostaglandin pathways.

Iben et al. (22) have previously shown under in vivo conditions that the response of total lung resistance to vagal stimulation of rat pups is significantly potentiated in normoxic animals by prior blockade of NOS. These observations mirror our findings under in vitro conditions. Iben et al. also showed under in vivo conditions that NOS blockade with 1-NAME in hyperoxic animals did not result in further enhancement of total lung resistance responses to vagal stimulation compared with normoxic animals. In our study, NOS blockade under in vitro conditions impaired airway relaxation of preconstricted trachea from both hyperoxic and normoxic rat pups. One possible reason for this discrepancy is that diminished relaxant responses and cGMP production in the hyperoxic vs. normoxic groups did not reach statistical significance because of relatively small sample sizes and the large range of responses observed between groups of rat pups. A more likely possibility may relate to differential effects of hyperoxic exposure on release of endogenous NO at different sites. Under in vivo conditions, lung resistance measurement includes resistance generated from large and small airways, as well as possibly tissue contractile and vascular elements. In our present study, we deliberately conducted both physiological and biochemical studies exclusively on trachea to correlate biochemical and physiological data derived from airway structures, and relate them to the resultant contractile and relaxant responses of airway smooth muscle. Future studies should incorporate measurements of NO release from airway segments, because recent data indicate that expression of NOS in airway epithelium is attenuated in the fetal baboon model of bronchopulmonary dysplasia (2).

Interaction between prostaglandins and NO has been described previously in several tissues (1, 18, 19, 29, 30). In rat pups, our laboratory has previously described a potential interaction between NO and prostaglandins in SP-induced airway relaxation (30). In our present study, it is difficult to determine whether there is any interaction between the NO-cGMP and PG-cAMP pathways. Future studies measuring cAMP and cGMP in the presence of selective NOS and/or cyclooxygenase inhibitors are needed to determine whether there is any such interaction.

We found in our present study that, under baseline conditions before EFS, tracheal cAMP and PGE\textsubscript{2} are higher in hyperoxic than normoxic rat pups. This increase in baseline content during hyperoxia may be related to the increase in PGE\textsubscript{2} release before EFS. Our laboratory has previously observed an increase in SP in lungs of hyperoxia-exposed rat pups (3). This excess of SP in the lungs of hyperoxic rat pups may also have contributed to the increase of PGE\textsubscript{2} and cAMP at baseline before any stimulation. This

Table 2. cGMP levels in trachea of normoxic and hyperoxic rats at baseline and after EFS

<table>
<thead>
<tr>
<th></th>
<th>Baseline (n=8)</th>
<th>30 s (n=8)</th>
<th>60 s (n=8)</th>
<th>180 s (n=8)</th>
<th>P Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normoxia</td>
<td>79.8±22.3</td>
<td>34.1±9.9</td>
<td>89.9±17.4</td>
<td>351.5±180.0†</td>
<td>0.08</td>
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<tr>
<td>Hyperoxia</td>
<td>89.3±26.4</td>
<td>29.3±5.0</td>
<td>75.8±16.4‡</td>
<td>185.0±68.0</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Values are means ± SE given in fmol/μg of protein; n, no. of animals. *One-way ANOVA comparing levels of cGMP over time (from baseline to 180 s) in tracheae from normoxic or hyperoxic animals. †Post hoc analysis demonstrating significant increase of cGMP at 180 s vs. baseline (normoxic group; P < 0.05). ‡Post hoc analysis demonstrating a significant increase of cGMP at 60 s compared with at 30 s but not at baseline (P < 0.05).

Table 3. Prostaglandin E\textsubscript{2} levels from trachea of normoxic and hyperoxic rats at baseline and after EFS

<table>
<thead>
<tr>
<th></th>
<th>Baseline (n=9)</th>
<th>60 s (n=9)</th>
<th>180 s (n=9)</th>
<th>P Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normoxia</td>
<td>117.9±4.3</td>
<td>126.0±1.3</td>
<td>134.1±2.8</td>
<td>0.004</td>
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<tr>
<td>Hyperoxia</td>
<td>130.7±3.0†</td>
<td>135.5±2.9</td>
<td>139.4±4.0</td>
<td>0.21</td>
</tr>
</tbody>
</table>

Values are means ± SE given in pg/ml of tissue supernatant; n, no. of animals. *One-way ANOVA comparing levels of prostaglandin E\textsubscript{2} over time (from baseline to 180 s) in tracheae from normoxic or hyperoxic animals. †Post hoc demonstrating significantly elevated levels of prostaglandin E\textsubscript{2} at baseline between tracheae from normoxic and hyperoxic animals (P = 0.02).

Fig. 5. The percent change in cGMP production over time, after EFS stimulation, was similar between normoxic and hyperoxic animals. Each data point represents a mean ± SE derived from trachea of 8 different animals (2-way ANOVA, P = 0.26).
would be consistent also with our previous observation that SP induces airway smooth muscle relaxation via PGE$_2$ (30). Other potential mechanisms for increased levels of cAMP in the trachea of hyperoxic animals may relate to decreased phosphodiesterase activity after hyperoxic exposure. Future studies will, therefore, need to elucidate the role of phosphodiesterase as well as cyclooxygenase activities (including cyclooxygenase-1 and cyclooxygenase-2) in the trachea of immature animals exposed to hyperoxia.

There was no difference in the percent relaxation to exogenous PGE$_2$ exposure between tracheae from normoxic and hyperoxic animals in our study, which supports the concept that deficient release of prostaglandin in response to EFS is a major mechanism for impaired relaxation in hyperoxia-exposed airways. We have not directly measured PGE$_2$ and cAMP accumulation from tracheal segments incubated with indomethacin before EFS, and this needs evaluation in future studies. The present data do not allow us to further speculate as to the mechanism contributing to the impairment in PGE$_2$ release and cAMP generation under our experimental conditions. Prior studies in rat pups exposed to hyperoxia at 3 wk of age have demonstrated significant reduction in thickness of tracheal epithelium (21), although our laboratory has not previously observed consistent anatomic changes in the trachea of rat pups exposed to hyperoxia at day 7 (3). In neonatal guinea pigs, hyperoxic exposure also does not appear to elicit histological changes in airway structures (38). Nonetheless, future studies might correlate anatomic changes in epithelial structures with the functional changes we have observed to coincide with hyperoxic exposure in early life. The present study also did not seek to selectively characterize the various neural pathways that might contribute to airway relaxant responses in rat pups. For example, failure to eliminate relaxant responses of tracheal segments with indomethacin and l-NAMe suggests a role for other cotransmitters such as vasoactive intestinal peptide, which may also be vulnerable to interventions in the neonatal period (7). Future studies are needed to determine the effect of hyperoxia on these relaxing pathways by using adrenergic and cholinergic blockade as well as tetrodotoxin to establish the nature of the relaxant response measured and the interaction between different relaxing substances and the PGE$_2$-cAMP pathway.

Conclusion. The results of this study indicate, for the first time, that hyperoxia impairs smooth muscle relaxation of large airway in immature animals mainly through alteration of the PGE$_2$-cAMP signaling pathway. This may contribute to the enhanced airway reactivity in hyperoxia-exposed neonates. Future studies elucidating the mechanisms whereby PGE$_2$-cAMP production is impaired during hyperoxia may help to enhance our understanding of the pathophysiology of chronic lung disease in premature infants.

GRANTS

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