Lipid Mediators in Oxygen-induced Airway Remodeling and Hyperresponsiveness in Newborn Rats

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We examined whether lipid mediators have a causal role in neonatal hyperoxia-induced lung damage, specifically, airway remodeling and hyperresponsiveness. Newborn rat pups were exposed to hyperoxia (> 95% O_2 from Days 4 to 14 and 65% from Days 14 to 32) or normoxia. The 5-lipoxygenase inhibitor, LTD_4 receptor antagonist, and inhibitor of platelet-activating factor synthesis, Wy-50,295 (30 mg/kg), or vehicle was administered daily from Days 3 to 32. Oxygen exposure significantly increased ($p < 0.05$) the production of one potential lipid mediator group, peptido-LTs, from explanted lung slices and large airways from 2-wk-old rat pups. At 4 wk, only the large airway tissue output showed significant elevation because of oxygen exposure. At both ages, Wy-50,295 significantly decreased ($p < 0.05$) the production of peptido-LTs in the lung and large airways of oxygen-exposed pups. Pulmonary function and airway wall morphometry were studied in 5-wk-old rat pups 2 to 3 d after oxygen exposure and drug administration ceased. The resistance change in response to methacholine (0 to 20 μg/kg body weight given intravenously) was greater ($p < 0.02$) in oxygen-exposed animals. Oxygen exposure caused significant (60% increase) smooth muscle thickening ($p < 0.05$). Wy-50,295 prevented the oxygen-induced airway hyperresponsiveness and smooth muscle thickening. We conclude that chronic hyperoxic exposure causes an increase in pulmonary production of at least one lipid mediator, peptido-LTs, from newborn rats and that this is associated with airway smooth muscle layer thickening and, consequently, airway hyperresponsiveness. Burghardt JS, Boros V, Biggs DF, Olson DM. Lipid mediators in oxygen-induced airway remodeling and hyperresponsiveness in newborn rats.

AM J RESPIR CRIT CARE MED 1996;154:837–42.

Bronchopulmonary dysplasia (BPD) is a multifactorial chronic lung disease of infancy that occurs after ventilator and oxygen therapy for neonatal respiratory distress. Hyperoxia is one of the major pathogenic factors of BPD, causing endothelial and epithelial cell damage. The typical histologic features of BPD include marked airway changes such as squamous metaplasia of airways and increased bronchial and peribronchial smooth muscle with fibrosis (1). Airway responsiveness, as defined by responsiveness to bronchoconstrictor challenge, is present within the first weeks of life and persists in adolescents with a history of BPD (1). Furthermore, in various animal models, airway hyperresponsiveness has been evoked by acute exposure to high oxygen concentrations (2, 3).

Although oxygen toxicity directly injures the neonatal lung, damage and impaired healing may be potentiated by the release of lipid mediators. These include the leukotrienes (LTs), LTB_4 and the peptido-LTs (C_4, D_4 and E_4), and platelet-activating factor (PAF). Elevated LT levels have been found in infants with BPD (4, 5). LTs are well-known mediators of human asthma and allergen-induced rodent asthma. Products of the 5-lipoxygenase pathway of arachidonic acid metabolism, particularly the LTs, can mediate bronchoconstriction, airway smooth muscle proliferation, and mucosal edema (6, 7). PAF has many actions that mimic those of peptido-LTs, including edema, mucus production, bronchoconstriction, and hyperresponsiveness (8). However, a possible role for lipid mediators in oxygen-induced lung damage in newborns has not been established.

The objective of this study was to characterize both the functional and morphologic changes of airways after chronic oxygen exposure during the newborn period and to study the association of these events with one potential lipid mediator, peptido-LTs, in hyperoxia-induced airway obstruction and hyperresponsiveness.

METHODS

Animals

Sprague-Dawley albino rat pups (Charles River Laboratories, St. Constant, Quebec, Canada) of both sexes were used. They were housed in the Health Sciences Laboratory Animal Service Department of the

(Received in original form July 10, 1995 and in revised form December 5, 1995)

Supported by The Alberta Heritage Foundation for Medical Research, the Medical Research Council of Canada, the Natural Sciences and Engineering Research Council of Canada, the Molly Towell Perinatal Research Foundation, the Alberta Lung Association, and the University of Alberta Central Research Fund.

Wy-50,295 was a gift from Wyeth-Ayerst Research, Princeton, NJ.

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University of Alberta under veterinary supervision. The guidelines of the Canadian Council of Animal Care were followed in all experimental procedures. Dams suckled the pups until they were 21 d of age. Dams and weaned pups were maintained on regular laboratory rodent pellets and water ad libitum and kept on a 12-h light/dark cycle.

Oxygen Exposure

Parallel litters of randomly divided rat pups were placed into 0.14-m^3 Plexiglas® exposure chambers containing > 95% or 21% O_2 at 4 d of life. O_2 levels were monitored daily (Ventronic O_2 analyser No. 5577; Hudson Co., Temecula, CA) and maintained at 95 to 100% until the pups were 14 d of age, when O_2 levels were lowered to 65%. Levels were maintained at 65% until Day 32. Oxygen and air were filtered through barium hydroxide lime, to keep CO_2 under 0.5%, and through charcoal. Temperature and humidity were maintained at 26° C and 75 to 80%, respectively. Chambers were opened for 15 min daily to switch dams between O_2 and air environments, change dirty cages, and administer the inhibitor/LT antagonist. On Day 32 pups were weaned from oxygen and the inhibitor/LT antagonist over a 48-h period before pulmonary function was measured or lungs were fixed for morphometric analysis.

LT Inhibition

To examine the effect of lipid mediator inhibition/LT antagonism, Wy-50,295 (30 mg/kg body weight; Wyeth-Ayerst Research, Princeton, NJ) or vehicle was administered subcutaneously to rat pups from Days 3 to 14 of life and orally from Day 15 onward. The vehicle consisted of a mixture of 1 unit of Tween 80 (Sigma, St. Louis, MO) with 4 units of distilled H_2O for subcutaneous administration or 1 unit of Tween 80 with 4 units of 1.5% methylcellulose (Sigma) for oral administration. This resulted in four experimental groups: air (21% O_2) + vehicle, air + Wy-50,295, O_2 (> 95% from Days 4 to 14 and 65% for the remainder of exposure) + vehicle, and O_2 + Wy-50,295. This dose of Wy-50,295 was selected because preliminary trials proved it to effectively inhibit peptido-LT output from parenchymal lung slices *in vitro* when administered subcutaneously (*see below*). Tissue levels of the drug were not determined, but this concentration (if evenly distributed without metabolism or excretion) would inhibit both 5-lipoxygenase and PAF acetyl transferase activities.

Peptido-LT Production

Lung and airway peptido-LT production was measured using a short-duration lung explant technique (9). Briefly, pups were killed with an overdose of pentobarbital (100 mg/kg Euthanyl™; MTC Pharmaceuticals, Cambridge, Ontario, Canada). The distal trachea with the extrapulmonary part of the main bronchi or three blood-free lung slices 500 μm thick (sliced using a tissue slicer from Stoelting Co. (Wood Dale, IL) were placed in tissue culture wells (12-well plate; Costar, Cambridge, MA) containing 800 μl of tissue culture medium (Hanks buffered salt solution buffered with HEPES to pH 7.36, 1.67 mM $CaCl_2$). After a 30-min incubation at 37° C the peptido-LT concentration of tissue culture medium was measured using a peptido-LT ELISA kit (Oxford Biomedical Research Ltd., Oxford, MI). The kit contains LTC_4 standard, LTC_4-horseradish peroxidase enzyme conjugate, and monoclonal rat $LTC_4/LTD_4/LTE_4$ antibody. The cross-reactivity of the kits used were 100% for LTC_4, > 80% for LTD_4 and LTE_4, < 2% for LTA_4 and < 1% for LTB_4. Peptido-LT concentrations were normalized to the total DNA content of the tissue (10).

Pulmonary Function

Pulmonary resistance (RL) was measured in 5-wk-old anesthetized (urethane, 1.0–1.75 mg/kg; Sigma), ventilated rat pups. The trachea was cannulated with polyethelene tubing 2.5 cm long (Intramedic PE 205; Becton Dickinson, Rutherford, NJ). Artificial ventilation was accomplished by attaching the cannula to a modified Harvard small-animal respiration pump (tidal volume, 10 to 12 ml/kg; 40 breaths/min) (Harvard Apparatus Co., South Natick, MA). The bronchoconstrictor, methacholine (0.2 to 20 μg/kg in physiologic saline), was administered intravenously via a 24-gauge, three-quarter-inch catheter (Insulyte-W®; Becton Dickinson) inserted into the femoral vein. Resistance values were measured using the computer-directed View-Dac™ data acquisition system, which computes the resistance from the flow rate and pressure (11).

After tracheal cannulation and intravenous catheterization, the spontaneously breathing animals were attached to the ventilator. Baseline RL was determined after saline was administered and was calculated from the mean of 10 breaths. Then the first dose of methacholine (0.2 μg/kg) was administered, and the peak RL value was recorded. The animal was removed from the ventilator approximately 30 to 40 breaths after administration of the dose and was allowed to recover for 2 min before the procedure was repeated again with the next methacholine dose. Resistance changes after methacholine injections were expressed as the percentage of the peak resistance relative to the baseline immediately before methacholine injection [(peak RL/baseline RL) × 100%]. A dose-response curve was generated for each animal, and the EC_{200} was calculated. The EC_{200} is the concentration of methacholine required to increase RL to 200% of the value measured after saline administration (baseline) (12). We considered baseline values to be 100%.

Extravascular Lung Water

Extravascular lung water (ELW) was assessed by calculating wet/dry lung weight ratios. Briefly, lungs were removed from the chest cavity and blotted minimally, and wet weights were determined. The lungs were then allowed to dry in a 37° C oven and were weighed daily. The tissue was considered dry when the weight was consistent for 2 consecutive days (usually 3 d later), and dry weight was recorded.

Airway Morphometry

Lungs of 5-wk-old rat pups were fixed *in situ* via a tracheal cannula by inflation with 2.5% glutaraldehyde at a constant inflation pressure of 20 cm H_2O for 2 h and then excised. The trachea was ligated, and the lungs were immersed in glutaraldehyde and fixed for an additional 24 h. After embedding in paraffin, cross sections of left and right lungs were cut and the slices were stained with Gomori trichrome and aldehyde fuchsin.

Airway layer fractional areas were identified and calculated by an image-analysis system consisting of a microscope (Carl Zeiss Variant Jenamed), video camera (MITS 68), computer (386 Modular PC; IBM), and image-analysis software (Genias 25; Joyce-Loebl Co., England). Airways cut obliquely, as defined by a circularity ($4\pi \times$ area/perimeter^2) below 0.7 or above 1.3, were eliminated from analyses. The resulting number of circularly cut small airways averaged 10 per animal. Only small airways with a circumference less than 500 μm, as defined by the total airway wall perimeter, were measured in this study.

Airways projected from the microscope to the computer screen consisted of a lightly stained epithelial layer (EL), a dark elastin band located beneath the EL, and a smooth muscle layer (SML) contaning smooth muscle cells, extracellular collagen, and elastin. The measurement for each airway was accomplished in two steps. The area (A) of the total airway wall (TW) consisting of EL and SML was measured first. The epithelial basement membrane (BM) was traced with a light pen, separating the EL from the SML. AEL was then measured. ASML was calculated as ATW − AEL. Each area measurement was normalized to the length of the epithelial BM.

Statistics

Data from methacholine dose-response curves were analyzed for significant differences by using repeated measures ANOVA. For all other measurements, 1-factor ANOVAs were used. To determine differences between the individual groups, Fisher's projected least-significant-difference post hoc test was used. All data are expressed as mean ± SD, except in Figure 3 where SEM is used to improve the clarity of the figure.

RESULTS

Peptido-LT Production

Peptido-LT production from lung and large airway tissue is shown in Figures 1 and 2. After 30 min of incubation, peptido-LT production was significantly ($p < 0.05$) elevated in the O_2 + vehicle group from both lung and airway tissue from 2-wk-old rat pups (Figures 1A and 2A). Meanwhile, at 4 wk, only the airway tissue peptido-LT output showed significant ($p < 0.05$) elevation (Figure 1B) because of O_2 exposure. The lung tissue peptido-LT

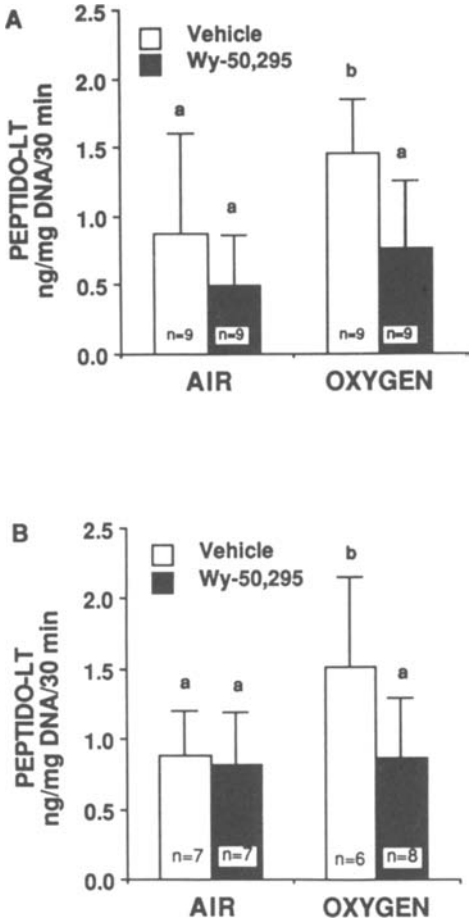

Figure 1. Effect of oxygen exposure and LT inhibitor Wy-50,295 on large airway (extrapulmonary airways) peptido-LT production of 2-wk-old (*A*) and 4-wk-old (*B*) rat pups. Oxygen exposure increased LT production at both ages. Wy-50,295 significantly reduced LT production of oxygen-exposed rat pups. The data are expressed as mean ± SD. Different letters = $p < 0.05$ (n = 7 to 9).

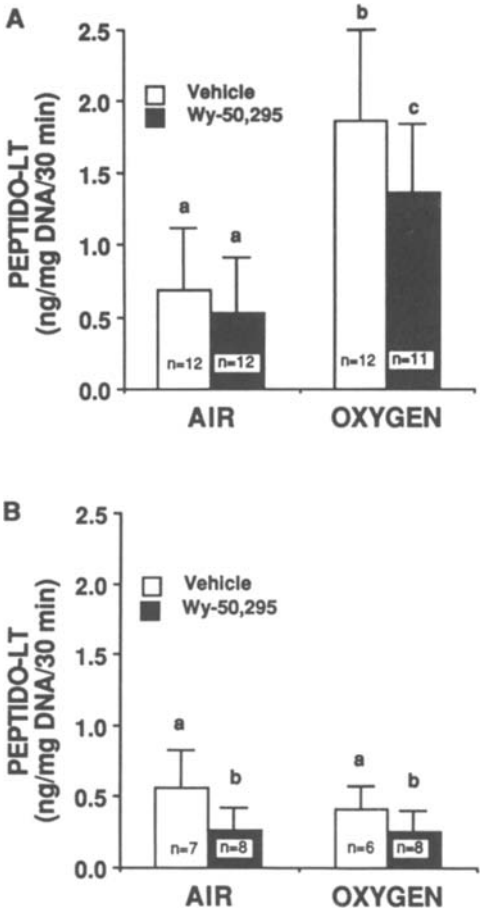

Figure 2. Effect of oxygen exposure and LT inhibitor Wy-50,295 on lung peptido-LT production of 2-wk-old (*A*) and 4-wk-old (*B*) rat pups. At 2 wk, oxygen exposure significantly increased LT output. Wy-50,295 significantly diminished LT production of lung tissue. The data are expressed as mean ± SD. Different letters = $p < 0.05$ (n = 6 to 12).

output from the O_2 + vehicle group was similar to the air + vehicle group (Figure 2B).

At both ages Wy-50,295 significantly ($p < 0.05$) inhibited the production of peptido-LTs in the large airways of oxygen-exposed animals. The levels from the O_2 + Wy-50,295 groups were similar to those from the air + vehicle group. Levels were decreased in air-exposed animals; however, they were not found to be statistically significantly different from the air + vehicle group (Figure 1A and B).

At 2 wk, Wy-50,295 was able to decrease the production of peptido-LTs from lung tissue of oxygen-exposed animals but not to normal (air + vehicle) levels. As with the airway, Wy-50,295 was able to decrease peptido-LT levels at 2 wk of age, but not significantly. In 4-wk lung tissue, the peptido-LT production was not influenced by oxygen-exposure, i.e., production in the O_2 + vehicle group was equal to production in the air + vehicle group. Wy-50,295 significantly ($p < 0.05$) inhibited the LT production in lung tissue in both air and oxygen groups.

Pulmonary Function

Baseline R_L values are shown in Table 1. There was no significant difference in baseline R_L between any of the groups, although the value for the O_2 + vehicle group was somewhat higher than in the others.

Oxygen-exposure caused a significantly ($p < 0.05$) higher airway response to methacholine in 5-wk-old rat pups, as evidenced by a higher resistance change at 2- to 20-μg/kg doses (Figure 3). Administration of Wy-50,295 effectively prevented the formation of oxygen-induced airway hyperresponsiveness, whereas it had no effect in air animals. It can be seen in Figure 3 that the values for the O_2 + Wy-50,295 group were equal to the air

TABLE 1

EFFECTS OF OXYGEN AND LT INHIBITOR WY-50,295 ON BASELINE RESISTANCE (BR_L) AND EC_{200} VALUES OF 5-WK-OLD RAT PUPS*

Group	BR_L (*cm H_2O/ml/min*)	EC_{200}† (*μg/kg body weight*)
Air + vehicle	0.196 ± 0.083[a] (n = 23)	9.94 ± 3.45[a] (n = 23)
Air + Wy-50,295	0.197 ± 0.053[a] (n = 17)	9.14 ± 4.85[a] (n = 17)
Oxygen + vehicle	0.203 ± 0.062[a] (n = 18)	5.23 ± 4.37[b] (n = 18)
Oxygen + Wy-50,295	0.199 ± 0.057[a] (n = 16)	11.16 ± 4.26[a] (n = 16)

* Values are expressed as means ± SD. Different superscripts = $p < 0.05$.
† Concentration of methacholine required to raise R_L to 200% of baseline.

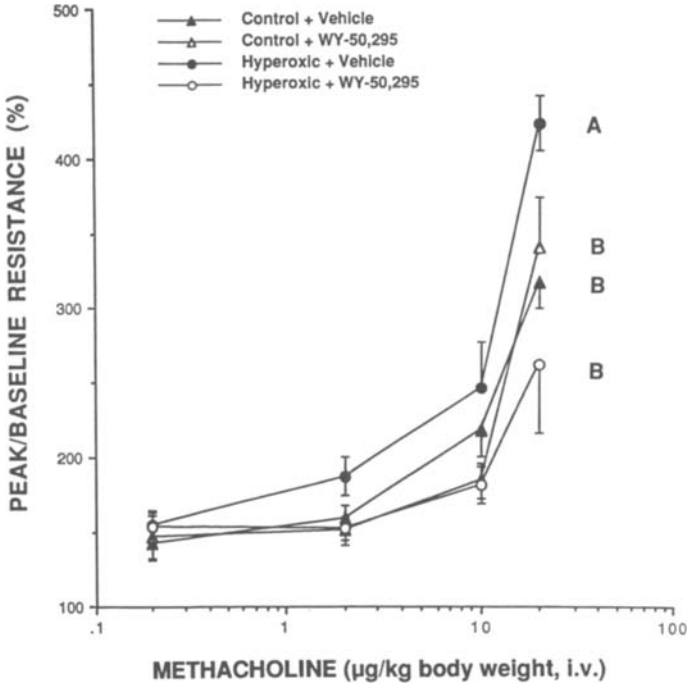

Figure 3. Effect of oxygen exposure and LT inhibitor Wy-50,295 on intravenously administered methacholine-induced airway constriction of 5-wk-old rat pups. Oxygen significantly ($p < 0.02$) increased the airway responsiveness to methacholine ($n = 18$) compared with air + vehicle ($n = 23$), air + Wy-50,295 ($n = 17$), and O_2 + Wy-50,295 ($n = 16$) groups. The data are expressed as mean ± SE. Different letters = $p < 0.02$.

+ vehicle and air + Wy-50,295 groups. The same trend was evidenced by the EC_{200} values (Table 1). EC_{200} was significantly ($p < 0.05$) lower in the O_2 + vehicle group than in all the other groups.

ELW

To determine the possible contribution of edema to airway obstruction, ELW was measured. The lungs of oxygen-exposed

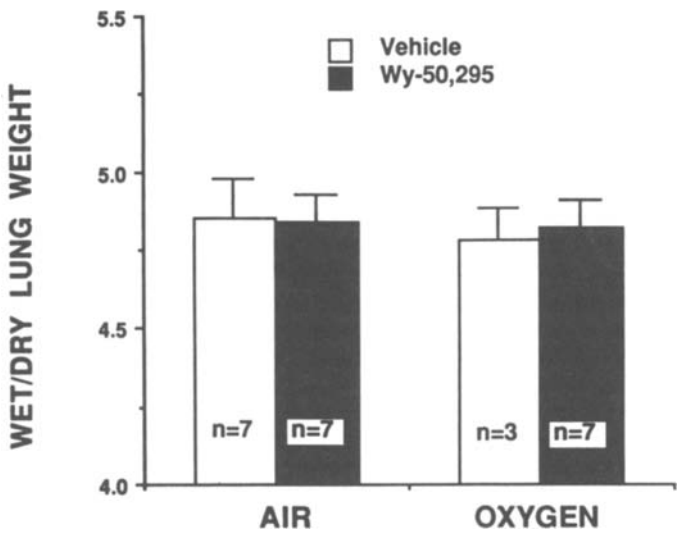

Figure 4. Effects of oxygen exposure and LT inhibitor Wy-50,295 on wet/dry lung weight ratios of 4-wk-old rat pups. There was no difference between any of the groups.

TABLE 2

EFFECTS OF OXYGEN EXPOSURE AND LT INHIBITOR WY-50,295 ON AIRWAY WALL MORPHOMETRY OF 5-WK-OLD RAT PUPS*

Group	AEL (μm^2) / LBM (μm)	ASM (μm^2) / LBM (μm)	ATW (μm^2) / LBM (μm)
Air + vehicle	0.684 ± 0.026[a]	0.432 ± 0.061[a]	1.116 ± 0.084[a]
Air + Wy-50,295	0.650 ± 0.027[a]	0.380 ± 0.071[a]	1.030 ± 0.095[a]
Oxygen + vehicle	0.697 ± 0.027[a]	0.691 ± 0.106[b]	1.388 ± 0.095[b]
Oxygen + Wy-50,295	0.669 ± 0.078[a]	0.481 ± 0.092[a]	1.166 ± 0.159[a]

Definition of abbreviations: AEL = epithelial layer area; ASM = smooth muscle layer area; ATW = total wall area (AEL + ASM); LBM = epithelial basement membrane length.

* Values are expressed as means of seven to 10 airways/rat for four rats ± SD. Different superscripts = $p < 0.05$.

animals were not edematous at 4 wk of age, nor was there any effect caused by administration of Wy-50,295, as evidenced by equal wet/dry lung weight ratios between all four groups (Figure 4).

Airway Morphometry

Airway morphometric data of 5-wk-old rat pups are shown in Table 2. Oxygen exposure significantly increased the ATW ($p < 0.05$) and ASML ($p < 0.001$) compared with all other groups. Meanwhile AEL was not affected by oxygen exposure. In O_2 + Wy-50,295 pups, ATW and ASML were equal to the corresponding areas of both air groups.

DISCUSSION

Follow-up studies of infants with BPD have shown that airway hyperresponsiveness persists well into adolescence (13–19). In acute animal studies of relatively short-term exposures, newborns exposed to high levels of oxygen develop increased airway reactivity. In an acute exposure (85% O_2 for 84 h), 1- to 2-d-old guinea pigs exhibited increased airway reactivity to acetylcholine without changes in airway histology (2). In contrast, our study of sustained or chronic exposure had newborn rat pups exposed to > 95% O_2 from Days 4 to 14 and 65% from Days 14 to 32. Pulmonary function and morphometric measurements were obtained from animals 2 d after the cessation of hyperoxic exposure. This was done in an attempt to investigate the chronic effects of O_2, as opposed to its acute effects.

The methacholine dose-response curve for O_2 + vehicle rats was shifted to the left compared with that in the air animals, indicating the development of airway hyperresponsiveness. The lower EC_{200} values for the O_2 + vehicle group confirmed this. Methacholine provocation of airways was carried out only with intravenous administration of the agent and not combined with methacholine aerosol challenge. The distribution of the contractile agonist during intravenous and aerosol administration is likely to be different. We assumed that the distribution of agonist administered intravenously would be relatively homogeneous throughout the lung and therefore appropriate for small airway challenge. Nagase and colleagues (20) concluded that the intravenous administration of methacholine induced more modest, but relatively homogeneous, constriction of the bronchial tree and lesser degree of tissue distortion. Meanwhile, aerosol administration had a heterogeneous effect, and less airway constriction was observed as airways decreased in size. Because large airways proximal to airway generation 14 (21) with outer circumferences greater than 500 μm were not analyzed in the morphometric study, methacholine challenge of small airways was more relevant for us. The absence of edema in 4-wk-old rats, as indicated by similar wet/dry lung weight ratios and similar epithe-

lial layer thickness in oxygen and air animals, eliminated the possibility that increased R_L was due to airway obstruction caused by increased vascular permeability.

One of the possible causes for increased airway responsiveness seen in patients with BPD is airway smooth muscle thickening known to occur in infants with this affliction (22). In our study, morphometric analysis of small airways (< 500 μm circumference) showed some striking differences between the O_2 + vehicle group and the air group. Hyperoxic exposure caused a significant thickening of the smooth muscle layer. Whether this is due to hypertrophy or to hyperplasia of smooth muscle cells or an increase in collagen is unknown. Change in the amount of airway smooth muscle is a plausible explanation for increases in airway reactivity induced by hyperoxic exposure (2, 23).

Exposing 21-d-old rats to > 95% O_2 for 8 d increased the thickness of the epithelial layer (23). We found no differences in airway epithelial thickness in our study. This may stem from the fact that our model is one of long-term hyperoxic exposure, and it is possible that epithelial layer repair occurred before morphometric analysis was accomplished. Such evidence exists in 21-d-old rats exposed to 95% O_2 for 8 d where airway repair occurs gradually from the time the animals are removed from oxygen. In fact, epithelial repair occurs at a much faster rate during the first 3 d after oxygen exposure than repair in the smooth muscle layer, which does not change at all during this period (23).

In the search for mechanisms that translate the chronic effects of oxygen to altered airway smooth muscle development and subsequent physiologic changes, it is logical to explore the potential roles of lipid mediators. Peptido-LTs are potent bronchoconstrictors and inducers of mucous secretion and vascular permeability. They are found in high concentrations in the lavage fluid of infants with BPD (4, 24) and in the blood, BAL, and urine of asthmatics (25). In our model, hyperoxic exposure caused an increase in the production of peptido-LTs by the lung and large airway explants at 2 wk, and these levels remained elevated only in the large airways at 4 wk of age.

Although measuring the large airway explant peptido-LT production can give us insight into large airway synthesis, it does not necessarily reflect synthesis by the small airways. Not only is the cellular composition much different, but, presumably, the cellular interactions of the parenchyma influence the production of peptido-LTs by small airways. In an attempt to approach this issue, the output of peptido-LTs from lung parenchymal slices was studied. There were indeed differences in peptido-LT output between large airway explants and lung parenchymal slices, especially at 4 wk. At 2 wk, hyperoxia enhanced LT output from each type of preparation. However, at 4 wk only the large airways responded to hyperoxia by increasing peptido-LT output. One interpretation of these data may be that nonairway tissue of the parenchyma may have a different time course of responsiveness to hyperoxia. More detailed studies at the cellular level are required to sort out these specifics of production. Regardless, it is abundantly clear that peptido-LT production in response to hyperoxia is enhanced and is prolonged sufficiently to affect pulmonary development.

Our intention in this study was to draw a correlation between lipid mediators and their potential in affecting certain aspects of hyperoxic lung disease in newborn rats. We did not measure PAF, LTB_4, or individual peptido-LT levels in this study as it was beyond its scope to define specific mediators. Further investigation using more specific antagonists or synthesis blockers along with determinations of mass levels of specific lipid mediators is required to elucidate which mediators most probably alter lung development. Nevertheless, this study does suggest the possibility that peptido-LTs mediate oxygen-altered lung development. The data show that inhibition of LTs by Wy-50,295 prevents the development of oxygen-induced airway hyperresponsiveness and airway smooth muscle layer thickening.

Wy-50,295 is a potent 5-LO inhibitor with an inhibitory action that does not involve antioxidant mechanisms. Its eicosanoid selectivity has been demonstrated by its lack of effect against 12-LO, 15-LO, cyclooxygenase, and phospholipase A_2 enzymes (26). Wy-50,295 inhibited peptido-LT production of the large airway of oxygen-exposed rats and reduced peptido-LT levels to that of air animals at both 2 and 4 wk. The inhibition was less efficient in the lung explants at 2 wk. The levels were significantly lower than in O_2 + vehicle animals, but they were not as low as those in air animals. At 4 wk, Wy-50,295 significantly reduced peptido-LT production to below normal air values. Generally, Wy-50,295 had no significant effect on peptido-LT production in air animals, except in the lung slices at 4 wk. Hence, LT output from lung tissue correlates with the changes (or lack of changes) we have observed.

However, Wy-50,295 also inhibits PAF production by rat peritoneal mast cells (27). And, although Wy-50,295 is 12-fold more effective as an LT inhibitor than a PAF synthesis inhibitor (26, 27), it may be inhibiting PAF production at the concentrations we used. Therefore, further studies are required to identify the specific lipid mediators.

The concept that chronic exposure to lipid mediators may alter airway development is novel. Little is known, for instance, about the effects of peptido-LTs on airway smooth muscle over the long term. Our study shows that hyperoxia induces increased large airway peptido-LT production, which is sustained over the period of exposure. Airway smooth muscle has receptors for peptido-LTs, and LTD_4 receptor antagonists have been shown to eliminate LTD_4-induced airway thickening as well as hyperresponsiveness in asthma models (28–30). However, it may be that peptido-LTs work indirectly by stimulating synthesis of other mediators such as growth factors or cytokines that may more directly influence the smooth muscle mass.

Alternatively, lipid mediators may employ other mechanisms such as causing a chronic contraction of the airways, leading to hypertrophy. It was shown that exposing cultured rat tracheal smooth muscle cells to 70% O_2 resulted in increased smooth muscle cell protein content compared with cells exposed to 21% O_2 (31). Smooth muscle cell proliferation was inhibited by O_2 in the same study, suggesting that O_2 induced hypertrophy. Lipid mediators may play a role in maintaining pathologic tone in asthmatics. Also, human bronchial smooth muscle exhibits a high level of intrinsic tone that is due to continual production and release of peptido-LTs (32). In our study, baseline R_L was higher in the O_2 + vehicle group than in all the other groups, but the difference was not statistically significant. This suggests a greater tone in oxygen-exposed animals.

We conclude that chronic hyperoxic exposure causes an increase in one lipid mediator, peptido-LT, production in the lung of newborn rats and that this is associated with airway smooth muscle layer thickening and, consequently, the development of airway hyperresponsiveness. Further studies to elucidate the sources, nature, and specific actions of the lipid mediators are warranted.

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