Failure of Premature Rabbits to Increase Lung Antioxidant Enzyme Activities after Hyperoxic Exposure: Antioxidant Enzyme Gene Expression and Pharmacologic Intervention with Endotoxin and Dexamethasone

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ABSTRACT

Premature rabbits, unlike full-term rabbits, are unable to mount a protective increase in pulmonary antioxidant enzyme (AOE) activities in response to 48 h of hyperoxic exposure and demonstrate increased pulmonary O₂ toxicity compared with full-term rabbits. To examine AOE gene expression of CuZn superoxide dismutase (SOD), Mn SOD, catalase, and glutathione peroxidase in preterm *versus* term rabbits in response to hyperoxia, 29.5 d preterm rabbits (delivered by hysterotomy) and term rabbits (spontaneously vaginally delivered) were exposed to 48 h of >90% O₂ or room air. Preterm rabbits had a significant increase in CuZn SOD mRNA without corresponding AOE activity increases, suggesting translational/posttranslational inhibition. In full-term rabbits, the magnitude of lung AOE mRNA changes was associated with concordant magnitude changes in activities of CuZn SOD, Mn SOD, and catalase, suggesting pretranslational regulation of AOE gene expression; glutathione peroxidase, however, appears to be regulated translationally/posttranslationally. To investigate potential pharmacologic means of overcoming the susceptibility of the preterm rabbit to O₂ toxicity, 29.5 d preterm rabbits received 20–40 µg/kg of *Salmonella typhimurium* endotoxin or diluent S.C. (after birth and at 24 h); in separate experiments, pregnant rabbits received intramuscular injections of dexamethasone (0.01–0.05 mg/kg) or

saline on gestational d 27.5 and 28.5 and underwent hysterotomy at 29.5 d. After hyperoxic exposure, postnatal endotoxin treatment of preterm rabbits resulted in significant increases in CuZn SOD activity and CuZn SOD mRNA, suggesting a reversal of the translational/posttranslational inhibition characteristic of the preterm rabbit, improved hyperoxic survival (74/81 = 91% *versus* 70/92 = 76%, $p < 0.05$; endotoxin *versus* controls), and protection against hyperoxia-induced increases in lung lavage protein (+4% endotoxin *versus* +28% controls, $p < 0.05$). Prenatal dexamethasone neither improved hyperoxic survival, protected against O₂ toxicity, nor produced increases in any of the AOE after 48 h of hyperoxia relative to air-breathing preterm rabbits. (*Pediatr Res* 37: 469–475, 1995)

Abbreviations

AOE, antioxidant enzymes
SOD, superoxide dismutase
CuZn SOD, copper,zinc superoxide dismutase
Mn SOD, manganese superoxide dismutase
CAT, catalase
GP, glutathione peroxidase
DSPC, disaturated phosphatidylcholine
cRNA, complementary RNA

The incidence of chronic lung disease in human premature infants increases with decreasing gestational age (1) and is thought to be related in part to oxygen free radical-induced lung injury. In an attempt to explain the mechanisms involved with this chronic lung process and these epidemiologic observations, we have previously demonstrated that premature rabbits have decreased baseline activity levels of pulmonary AOE (2) and are also unable to mount a protective increase in AOE

activities in response to 48–72 h of hyperoxic exposure (3). Conversely, full-term newborn rabbits are able to significantly increase AOE activities with hyperoxia (3). Compared with term rabbits, which demonstrate minimal evidence of hyperoxic lung damage, preterm rabbits manifest increases in several indices of pulmonary O₂ toxicity, including lung lavage fluid protein content, lung conjugated diene levels, and light microscopic lung pathology (3).

We undertook the present study to investigate the molecular basis for the lack of hyperoxic AOE induction in preterm rabbits and its presence in term rabbits, and examined AOE gene expression after hyperoxic exposure at these two different

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gestational ages. In addition, to explore potential pharmacologic means of overcoming the susceptibility of the preterm rabbit to O₂ toxicity, we investigated whether, in the preterm rabbit, postnatal treatment with *Salmonella typhimurium* endotoxin or antenatal treatment with dexamethasone could reverse the early O₂ toxicity changes previously observed and/or reverse the inability of the preterm rabbit to induce a protective hyperoxic increase in pulmonary AOE activities. We also examined hyperoxic AOE gene expression after postnatal endotoxin or prenatal dexamethasone treatment.

METHODS

Animals

Timed-pregnant New Zealand albino rabbits were obtained from a local supplier (K-W Farms, Ft. Myers, FL), transported at least 1 week before term, and housed at the University of Miami Animal Care Facility. All animal care and experimental procedures were preapproved by the University of Miami Committee on Research Animal Welfare. To obtain preterm rabbits, pregnant rabbits were subjected to premature delivery at gestational d 29.5 by hysterotomy under ketamine/xylazine anesthesia (ketamine, 90 mg/kg; xylazine, 10 mg/kg i.v.), after which the abdominal vessels were cut to kill the animal. Twenty-nine and one half days of gestation was selected because at this gestational age, the animals still satisfy the definition of prematurity and demonstrate an inability to induce a protective hyperoxic AOE activity response, yet most are capable of surviving without vigorous supportive care including O₂ and mechanical ventilation (3). To obtain full-term newborn rabbits, pregnant rabbits were allowed to deliver spontaneously into breeder boxes at term (31–32 d).

The newly delivered 29.5 d rabbits were immediately resuscitated by drying, stimulation, 5–15 min of low flow O₂, and warming in 30–32°C isolettes. This process yielded initial survival of >90% of the preterm rabbits. Term rabbits were removed from their mothers within 18 h of delivery and placed into 30–32°C isolettes. Both the preterm and term rabbits were gavage fed via a 5 French feeding tube within 2 h of delivery for preterm rabbits and upon retrieval from their does for full-term rabbits and then at 24-h intervals with a simulated rabbit milk formula [consisting of casein hydrolysate (3%), whey protein (4.5%), corn oil (3%), coconut oil (3%), lactose (1%), complete vitamin and mineral mix (1%), and water to 100 mL, providing approximately 100 kcal/100 mL (US Biochemicals, Cleveland, OH, and ICN, Costa Mesa, CA) plus Mazola corn oil]. The volume administered was 6 mL/100 g body weight for the first feeding and 8 mL/100 g after 24 h. The diet enabled the preterm and newborn rabbits to maintain weight within 5–10% of birth weight but did not produce weight gain. However, newborn rabbits normally nurse once daily and do not gain body weight until after the third postnatal day (4).

For studies of AOE gene expression in preterm *versus* term newborn rabbits after hyperoxic exposure, 29.5-d preterm rabbits and spontaneously delivered term rabbits (who were removed from their mothers within 18 h of delivery) received no pharmacologic intervention, were weighed and numbered, re-

ceived their first artificial feeding, and were then randomized to either hyperoxia or room air exposure.

Pharmacologic Interventions

For the postnatal endotoxin studies, 29.5-d preterm rabbits were delivered by hysterotomy, resuscitated as described above, weighed and numbered, fed, and then randomly assigned to receive either postnatal endotoxin treatment or to serve as controls. Endotoxin was administered s.c. within 2–3 h of delivery and then at 24 h in the form of lipopolysaccharide (Westphal extracted) *S. typhimurium* (Difco Labs, Detroit, MI) at doses ranging from 20 to 40 µg/kg. Control preterm rabbits received equivolume s.c. injections of saline. The endotoxin-treated and control preterm rabbits were again randomly divided for subsequent exposure to either hyperoxia (>90% O₂) or room air for 48 h.

For the antenatal dexamethasone experiments, pregnant rabbits at 27.5 and 28.5 d of gestation received a once daily intramuscular injection of dexamethasone sodium phosphate (Steris Laboratories, Inc., Phoenix, AZ) at doses ranging from 0.01 to 0.05 mg/kg maternal body weight. Control pregnant rabbits received equivolume saline injections. After delivery and initial resuscitation, the 29.5-d preterm rabbits from mothers receiving either dexamethasone or saline were weighed and numbered, fed, and then randomly divided for exposure to either hyperoxia (>90% O₂) or room air for 48 h. In addition, a small number of randomly selected dexamethasone and control preterm rabbits were killed immediately after birth before air breathing to ascertain baseline AOE activities and lung DSPC content. In a separate experimental group of rabbits, rather than administering dexamethasone to the pregnant doe, we obtained preterm rabbits after hysterotomy delivery as described above, resuscitated them, and administered dexamethasone treatment postnatally within 1–2 h of delivery (and then 24 h later) at a dose of 5 µg/kg, and randomly assigned them to either >90% O₂ or room air exposure for 48 h.

Hyperoxia and Air Exposures

The 29.5-d preterm or term rabbits randomly assigned to hyperoxia were placed into 3.5-ft³ exposure chambers (adapted from infant isolettes; Air Shields, Hatboro, PA) and received >90% O₂ for 48 h; the chambers were monitored several times daily for gas concentration with SensorMedics (Dayton, OH) O₂ and CO₂ monitors. CO₂ was maintained at <0.4%. Those assigned to room air exposure were placed in similar chambers and received room air flow (21% O₂) for a 48-h period. Both groups received constant temperature (32°C), humidity (50–70%), and feeding conditions. Exposure to >90% O₂ or room air was continuous for 48 h, except for a daily 15- to 20-min period required for weighing, feeding, and cage cleaning (and for endotoxin experiments: endotoxin or saline injections).

Biochemical Analyses

After the 48-h hyperoxia or room air exposure period, the preterm or term rabbits were killed with an intraperitoneal overdose of pentobarbital. The abdominal vessels were cut to

exsanguinate the animals, the left atrial appendage was cut to facilitate drainage, and the lungs were perfused to whiteness via the pulmonary artery with cold saline. The lungs were then immediately frozen in liquid nitrogen and maintained at -70°C before biochemical analyses. Some preterm rabbits in the prenatal dexamethasone treatment group were killed (with an intraperitoneal overdose of pentobarbital administered through the unopened amniotic sac) before air breathing, and their lungs were perfused and then frozen as above for subsequent analysis of baseline AOE activities and lung DSPC.

AOE activity determination. For AOE activity analyses, thawed lungs were subsequently weighed, homogenized in cold saline (1:15 wt/vol) (Brinkmann Polytron, Westbury, NY), centrifuged for 45 min at 15 000 rpm, and then assayed by standard spectrophotometric techniques for activities of total SOD (xanthine/xanthine oxidase method) (5), CAT (rate of reduction of H_2O_2 at 240 nm) (6), and GP (rate of oxidation of NADPH at 340 nm using cumene hydroperoxide as substrate) (7). For the dexamethasone studies, only total SOD activity (in addition to CAT and GP) was measured. For determining CuZn SOD and Mn SOD activities, samples were desalted using a Bio Rad (Richmond, CA) Econo-Pac 10DG column and eluted in buffer containing 50 mM potassium phosphate plus 0.1 mM EDTA. Sample recovery was determined by comparing protein content (Coomassie Plus Protein assay, Pierce Co., Rockford, IL) in aliquots of samples before and after passage through the columns. After performing the xanthine/xanthine oxidase assay using both low concentrations (0.015 mM) and high concentrations (1.5 mM) of cyanide, CuZn SOD and Mn SOD activities were calculated as described by Iqbal and Whitney (8). Lung homogenates were also assayed for total DNA (9) and protein (10) content. Purified standards for SOD, CAT, DNA, and protein assays were obtained from Sigma Chemical Co. (St. Louis, MO); GP standard was obtained from Boehringer-Mannheim (Indianapolis, IN). AOE activity was expressed per mg of DNA.

AOE mRNA quantitation. Preparation of cRNA was performed as described previously (11, 12). Total nucleic acids were isolated from lung tissue according to published methodology (13). Samples of total nucleic acid solutions were then exposed to the ^{35}S -radiolabeled antisense cRNA probes for CuZn SOD mRNA, Mn SOD mRNA, CAT mRNA, and GP mRNA to quantitate these mRNA using solution hybridization (14). The unlabeled sense cRNA (of CuZn SOD, Mn SOD, CAT, and GP) were used as a standard to define the range in which hybridization of each radiolabeled probe increased pro-

portionately with added mRNA. Results of mRNA concentrations were expressed as specific mRNA molecules per mg of DNA.

DSPC analysis. Aliquots of lung homogenate were subjected to lipid extraction according to the method of Bligh and Dyer (15), reacted with osmium tetroxide as described by Mason *et al.* (16) to isolate the DSPC, and then assayed for inorganic phosphorus using the method of Morrison (17). A known quantity of ^{14}C -dipalmitoylphosphatidylcholine (New England Nuclear, Boston, MA) was added before lipid extraction to estimate and correct for sequential losses. DSPC was expressed as mg/g wet lung weight.

Indices of O_2 Toxicity

Daily survival was determined in both hyperoxia and room air exposure groups. In addition, randomly selected, unperfused right upper lobes of lungs from O_2 -exposed and room air-exposed preterm rabbits from the endotoxin and dexamethasone studies were subjected to wet lung weight/dry lung weight ratio measurements to roughly assess the effect of hyperoxia and specific pharmacologic interventions on lung water content. After the 48-h hyperoxia and room air exposure periods, as part of the endotoxin and dexamethasone experiments, randomly selected lungs were lavaged via a tracheal cannula with cold saline immediately after the rabbits were killed. Three lavages were performed using the volume necessary to fully inflate the right lung (the left lung had been clamped); the fluid was pooled and then measured for lavage protein content (10).

Statistical Analysis

Analysis of variance was used for comparing the four animal groups in each study. This was followed by Duncan's multiple range test with a p value < 0.05 used to indicate significant differences between the groups. Survival between groups was compared using χ^2 analysis (18).

RESULTS

AOE gene expression in preterm versus term rabbits. In association with the inability to increase lung AOE activities after 48 h of hyperoxic exposure (saline-control values, Tables 1 and 2), 29.5-d preterm rabbits demonstrated significant increases in CuZn SOD mRNA (+30%, $p < 0.05$) as well as elevations (which were not statistically significant) in Mn SOD

Table 1. AOE activities and lung DSPC after 48-h air or $>90\%$ O_2 exposure in endotoxin-treated and control 29.5-d preterm rabbits

Group	tSOD	CuZn SOD	Mn SOD	CAT	GP	DSPC
Endo-air	88.2 ± 30.6	82.7 ± 21.1	21.3 ± 7.6	146.9 ± 63.9	0.706 ± 0.246	6.79 ± 1.36
Endo- O_2	$104.4 \pm 29.1^*$	$98.8 \pm 23.5^*$	19.8 ± 8.1	162.5 ± 59.6	0.743 ± 0.226	6.47 ± 1.32
O_2 vs air	+18%	+19%	-7%	+11%	+5%	-5%
Cont-air	73.3 ± 22.8	69.6 ± 18.8	26.7 ± 4.3	138.6 ± 48.0	0.746 ± 0.168	5.44 ± 1.21
Cont- O_2	69.2 ± 21.3	72.5 ± 11.0	$21.6 \pm 4.0^*$	141.3 ± 65.1	0.760 ± 0.278	5.12 ± 0.86
O_2 vs air	-6%	+4%	-19%	+2%	+2%	-6%

Endo, endotoxin; Cont, control; tSOD, total SOD. Endotoxin: $n = 33$ –51 samples (lungs)/group. Control: $n = 10$ –33 samples (lungs)/group. Units: AOE, IU/mg DNA; DSPC, mg/g wet lung weight.

* $p < 0.05$ (O_2 values vs air values).

Table 2. AOE activities and lung DSPC after 48-h air or >90% O₂ exposure in dexamethasone-treated and control 29.5-d preterm rabbits

Group	tSOD	CAT	GP	DSPC
Prenatal dexamethasone				
Dexa-air	69.9 ± 25.5	128.6 ± 51.7	0.955 ± 0.475	5.39 ± 1.15
Dexa-O ₂	61.9 ± 29.7	115.0 ± 57.7	0.732 ± 0.290	5.36 ± 0.92
O ₂ vs air	-11%	-11%	-23%	0%
Postnatal dexamethasone				
Dexa-air	114.2 ± 33.7	228.3 ± 37.3	0.848 ± 0.019	6.40 ± 0.58
Dexa-O ₂	114.6 ± 12.7	248.0 ± 31.3	0.831 ± 0.081	7.01 ± 0.43
O ₂ vs air	0%	+9%	-2%	+9%
Saline controls				
Cont-air	78.8 ± 30.1	138.8 ± 39.9	0.682 ± 0.314	5.28 ± 0.91
Cont-O ₂	80.0 ± 31.0	140.5 ± 41.3	0.708 ± 0.290	5.08 ± 1.13
O ₂ vs air	+1%	+1%	+4%	-4%

Dexa, dexamethasone; Cont, control; tSOD, total SOD. Dexamethasone: Prenatal, $n = 27$ –30 samples (lungs)/group; postnatal, $n = 8$. Control: $n = 15$ –23 samples (lungs)/group. No differences are statistically significant.

mRNA (+14%), CAT mRNA (+19%), and GP mRNA (+23%) (Fig. 1A). (Numbers represent percent changes when 48-h hyperoxia-exposed lung AOE values are compared with 48-h air-exposed values.) Full-term rabbits exhibited a different

response to hyperoxia. In association with the significant increase in CuZn SOD activity (+31%, $p < 0.05$) after 48 h of hyperoxic exposure, full-term rabbits had a corresponding significant increase in CuZn SOD mRNA (+45%, $p < 0.05$).

(A) Antioxidant enzyme activity and mRNA response to hyperoxia: Preterm Rabbits

(B) Antioxidant enzyme activity and mRNA response to hyperoxia: Term Rabbits

(C) Antioxidant enzyme activity and mRNA response to hyperoxia: Endotoxin-treated Rabbits

(D) Antioxidant enzyme activity and mRNA response to hyperoxia: Dexamethasone-treated Rabbits

Figure 1. AOE activities (white bars) and corresponding antioxidant enzyme mRNA values (black bars) for preterm rabbits (A) ($n = 37$), term rabbits (B) ($n = 17$), endotoxin-treated preterm rabbits (C) ($n = 27$), and dexamethasone-exposed preterm rabbits (D) ($n = 20$). Values represent percent changes when 48-h hyperoxia values are compared with 48-h air values. For the dexamethasone-treated group (D) only, SOD activity represents total SOD activity; SOD mRNA represents CuZn SOD mRNA.

The changes in Mn SOD mRNA (+5%) and CAT mRNA (+14%), although not significant, were of a magnitude similar to that of the AOE activity changes (Mn SOD activity: +7%; CAT activity: +16.5%) (Fig. 1B). Despite the significant increase in GP activity (+32%, $p < 0.05$), GP mRNA was decreased 5% in term rabbits.

Endotoxin experiments. At the dosing range of 20–40 $\mu\text{g/kg/d}$, endotoxin-injected 29.5-d preterm rabbits demonstrated no significant differences in 48-h weights or in lung wet weight compared with saline control preterm rabbits. After a 48-h exposure period to >90% O_2 , postnatal endotoxin administration resulted in significant increases in activities of total lung SOD (+18%, $p < 0.05$) and CuZn SOD (+20%, $p < 0.05$) without producing significant changes in Mn SOD (–7%), CAT (+11%), or GP (+5%) activities (Table 1, Fig. 1C) in 29.5-d preterm rabbit lungs. (Numbers represent percent changes when 48-h hyperoxia-exposed lung AOE values are compared with 48-h air-exposed values.) During the same 48-h hyperoxic exposure period, saline-injected 29.5-d preterm rabbits demonstrated no increases in total SOD (–6%), CuZn SOD (+4%), CAT (+2%), or GP (+2%) activities and a significant decrease in Mn SOD activity (–19%, $p < 0.05$) (Table 1). No significant differences were present after 48 h of hyperoxia in lung DSPC content in either endotoxin-treated (–5%) or saline control (–6%) 29.5-d preterm rabbits (Table 1).

Endotoxin-treated preterm rabbits demonstrated significant increases in CuZn SOD mRNA (+31%, $p < 0.05$) but no significant increases in Mn SOD mRNA (+7%), CAT mRNA (+17%), or GP mRNA (+6%) compared with their AOE activity responses to hyperoxia (Table 1, Fig. 1C). The small, statistically insignificant changes in the CAT and GP mRNA were of a magnitude similar to that of the small changes in specific AOE activities after 48 h of hyperoxia (Fig. 1C).

In terms of indices of O_2 toxicity, endotoxin-treatment did not significantly influence survival after 48 h of air exposure (>90% survival for both preterm endotoxin-treated and controls). However, after 48 h of hyperoxic exposure, endotoxin-treated 29.5-d preterm rabbits demonstrated significantly improved survival compared with saline-injected controls (74/81 = 91% versus 70/92 = 76%, respectively; $p < 0.05$). Hyperoxia caused a significant increase in lung lavage fluid protein content in saline-injected preterm rabbits compared with air-exposed controls (+28%, $p < 0.05$). Endotoxin treatment protected against this toxic response to hyperoxia (lavage protein increase in hyperoxia-exposed versus air-exposed endotoxin-treated preterm rabbits, +4%). However, no differences were found between endotoxin and saline control groups in wet lung/dry lung weight ratios after 48 h of hyperoxic exposure.

Dexamethasone experiments. At the dosing range of 0.01–0.05 mg/kg maternal body weight, prenatal dexamethasone treatment resulted in a significant decrease in birth weight of 29.5-d preterm rabbits (–23%, $p < 0.05$) and a corresponding decrease in weights at 48 h, without any differences in lung wet weight compared with control preterm rabbits. Prenatal dexamethasone administration followed by a 48-h exposure to >90% O_2 resulted in no significant increases in activities of

total SOD (–11%), CAT (–11%), or GP (–23%) (Table 2, Fig. 1D) in 29.5-d preterm rabbit lungs. (Numbers represent percent changes when 48-h hyperoxia lung AOE values are compared with 48-h air values.) During the same 48-h hyperoxic exposure period, simultaneously exposed saline-injected 29.5-d preterm rabbits also demonstrated no increases in total SOD (+1%), CAT (+1%), or GP (+4%) activities. No significant differences were present after 48 h of hyperoxia in lung DSPC content (Table 2) in either dexamethasone-treated (0%) or saline control (–4%) 29.5-d preterm rabbits. Similarly, when dexamethasone-exposed 29.5-d preterm rabbits were compared with saline controls at the time of birth before air breathing, values for AOE activities and lung DSPC content were not different between dexamethasone and control preterm rabbits, respectively (total SOD: 35.5 ± 15.2 versus 39.3 ± 10.0 ; CAT: 49.0 ± 13.5 versus 42.2 ± 9.3 ; GP: 0.399 ± 0.124 versus 0.390 ± 0.077 IU/mg DNA; DSPC: 2.69 ± 0.47 versus 2.73 ± 0.84 mg/g wet lung weight). Finally, in the one experiment where dexamethasone was administered postnatally at a dose of 5 $\mu\text{g/kg}$, no significant increases were present after 48 h of hyperoxia in either AOE activities or lung DSPC content (Table 2).

Dexamethasone-treated preterm rabbits demonstrated no significant increase in CuZn SOD mRNA (+29%), CAT mRNA (+7%), or GP mRNA (+5%) (Fig. 1D) or in Mn SOD mRNA (7%) in association with their lack of hyperoxic increase in AOE activities (Table 2, Fig. 1D).

Dexamethasone-exposed preterm rabbits delivered at 29.5 d demonstrated decreased survival after 48 h of normoxic exposure (46/93 = 49% compared with 44/52 = 85% in controls, $p < 0.01$). Furthermore, dexamethasone-treated preterm rabbits did even less well after 48 h of exposure to >90% O_2 (44/126 = 35%) when compared with either normoxia-exposed dexamethasone preterm rabbits (46/93 = 49%, $p < 0.05$) or hyperoxia-exposed control preterm rabbits (52/71 = 73%, $p < 0.01$). However, the response to 48 h of hyperoxia was not different for dexamethasone versus control offspring in terms of lung lavage fluid protein content or in wet lung/dry lung weight ratios (data not shown).

DISCUSSION

Among the proposed etiologies for chronic lung disease in the preterm human infant are the immature development of the protective AOE system of the lung plus the inability to induce increases in pulmonary AOE activities to counteract the increased free-radical production associated with hyperoxic exposure. Although not yet definitively investigated in the preterm human infant, this phenomenon has been found in the preterm rabbit in our previous investigation (3) and confirmed in the present investigation. At 29.5 d of gestation, the newly delivered rabbit is unable to mount a protective hyperoxic AOE activity response and shows increased evidence of pulmonary O_2 toxicity compared with the full-term newborn rabbit. These findings for the preterm rabbit are consistent with findings in the premature baboon, a species that is also unable to increase AOE activities with hyperoxic exposure (19). However, a recent study from our laboratory has reported unexpectedly

different findings in the developing rat. Specifically, rats delivered 1 d prematurely had significantly increased activities of CuZn SOD, CAT, and GP and lung DSPC content (increases of a magnitude comparable to that in term rats exposed to hyperoxia), with increased survival rates in hyperoxia and decreased indices of O₂ toxicity (20).

To investigate the molecular basis for the lack of hyperoxic AOE induction in the preterm rabbit, the present study examined gene expression of the AOE after hyperoxic exposure in rabbits of preterm and term gestation. Although we had anticipated that the preterm rabbit's inability to increase AOE activities during hyperoxia would be related to a pretranslational defect in gene expression, our findings of a significant increase in CuZn SOD mRNA (with smaller, not statistically significant, increases in all AOE mRNA) without corresponding activity increases were unexpected and suggest translational/posttranslational inhibition of AOE gene expression in the preterm rabbit lung. For the term rabbit, we had anticipated increased AOE gene expression via pretranslational mechanisms, with increases in AOE mRNA associated with concordant increases in AOE activities. This phenomenon seemed to be present for CuZn SOD (activity: +30%, mRNA: +45%; both $p < 0.05$) and possibly CAT (activity: +16%, mRNA: +14%; neither increase was statistically significant). However, the significant increase in GP activity (+32%, $p < 0.05$) without increase in GP mRNA (−5%) suggests that GP activity changes may be regulated by a different mechanism, such as translational/posttranslational stimulation, or by a posttranscriptional mechanism involving increased rate of enzyme synthesis and/or increased stability of the enzyme. The finding that all of the AOE are not coordinately regulated in their gene expression responses to hyperoxia is consistent with previous developmental studies of the AOE in fetal and neonatal lungs (12, 20).

Bacterial endotoxin treatment (the complex lipopolysaccharide found in the cell wall of Gram-negative bacteria) enables adult rats, normally very O₂ intolerant and unable to increase AOE activities with hyperoxic exposure, to become significantly more oxygen tolerant and to demonstrate rapid lung AOE induction with hyperoxia. This response is regulated pretranslationally and consists of increases in both AOE activity levels as well as increases in AOE mRNA levels (13, 21–23). Based on these previous findings, we hypothesized that postnatal endotoxin administered to 29.5-d preterm rabbits would improve O₂ tolerance and induce hyperoxic increases in AOE mRNA that would then lead to increased pulmonary AOE activities. In fact, that is what our data clearly demonstrated for CuZn SOD. The endotoxin-treated preterm rabbits had significantly improved hyperoxic survival, evidence of decreased O₂ toxicity, and demonstrated significant increases in both CuZn SOD activity and mRNA, suggesting pretranslational regulation in response to hyperoxic exposure, similar to what was found for the untreated term rabbit. These findings also suggest that endotoxin reverses the translational/posttranslational inhibition of CuZn SOD gene expression characteristic of the preterm rabbit. One possible mechanism of this reversal by endotoxin may be through elevations in levels of specific cytokines such as tumor necrosis factor, IL-1, IL-6,

or platelet-derived growth factor; the synthesis of these cytokines has been demonstrated to be increased as a result of endotoxin (24).

Multiple lines of experimental evidence provided impetus for the hypothesis that glucocorticoids would enhance the maturation of the preterm rabbit lung and thereby reverse the inability to mount a protective hyperoxic AOE response. Prenatal dexamethasone treatment in the rat is associated with improved neonatal hyperoxic survival, decreased indices of O₂ toxicity, and greater and more rapid AOE responses to hyperoxia (25). Furthermore, glucocorticoids have been used clinically for acceleration of lung maturity in threatened premature delivery. The results of the present investigation in the preterm rabbit, in which antenatal glucocorticoids were unable to reverse the rabbit's inability to increase AOE activities with hyperoxia, were at variance with these experimental and clinical findings. A possible explanation for this discrepancy is species specificity of the positive effect of glucocorticoids on the developing lung, which although present in rat and human may be different in the rabbit. For example, rabbits seem to have increased sensitivity to the negative effects of low-dose prenatal glucocorticoids (*e.g.* growth retardation, decreased fetal survival) without manifesting evidence of enhanced lung maturation (26–29). This seemed to be the case in our present investigation (significantly decreased fetal weight and decreased 48-h normoxic survival but no evidence of enhanced lung maturity). There is also the possibility that dexamethasone failed to have any positive effect in our experimental rabbits due to the relatively late timing of treatments (gestational d 27.5 and 28.5). Evidence from other studies suggests that the peak “positive” maturational effect in the fetal rabbit lung may result from treatment at 25 and 26 d of gestation (30).

In conclusion, these results suggest that the inability of the preterm rabbit to induce a protective hyperoxic AOE activity increase is related to translational/posttranslational inhibition of AOE gene expression, a mechanism different from the pretranslational control present in the full-term rabbit. Postnatal endotoxin treatment (but not dexamethasone administration) seems to partially protect against O₂ toxicity and to reverse the translational/posttranslational inhibition of AOE gene expression characteristic of the preterm rabbit.

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