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Antioxidant Enzyme Responses to Hyperoxia in Preterm and Term Rats after Prenatal Dexamethasone Administration

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ABSTRACT. Although prenatal steroid therapy is known to enhance *in utero* maturation of the surfactant and antioxidant enzyme systems, little is known about the effects of steroids on the antioxidant system after birth. We measured activities of the antioxidant enzymes, catalase, superoxide dismutase, and glutathione peroxidase, in lung homogenates from both preterm and term rat pups after prenatal dexamethasone treatment. Enzyme activities were measured at birth and after exposure to >98% oxygen. Dexamethasone treatment resulted in significantly higher survival of the preterm pups at 24 h (91.3% for dexamethasone *versus* 57% for saline). In preterm pups, the activities of catalase and superoxide dismutase at birth were higher after dexamethasone treatment ($p < 0.05$). However, after 24 h of hyperoxic exposure, there were no differences in activities of any of the antioxidant enzymes between the dexamethasone and control groups of prematurely born pups. In term pups, antioxidant enzyme activities did not differ significantly at birth; nor did they differ after 24 to 72 h of hyperoxic exposure in the dexamethasone and control treatment groups. Our results indicate that although prenatal dexamethasone treatment augments survival and catalase and superoxide dismutase activities at birth in preterm rat pups, dexamethasone does not result in altered early postnatal antioxidant enzyme activities after exposure to hyperoxia. (*Pediatr Res* 33: 177–180, 1993)

Abbreviations

CAT, catalase
SOD, superoxide dismutase
GPX, glutathione peroxidase

The antioxidant enzyme system serves to protect the organism from O_2-free radicals that are produced as a result of breathing and metabolizing ambient O_2. Production of free radicals increases after exposure to hyperoxic conditions (1), and oxygen tolerance is thought to depend primarily on the capacity to augment antioxidant enzyme activities in response to hyperoxia (2–5). This capacity to increase antioxidant enzymes may account for the greater O_2 tolerance of term neonatal animals of several species compared with adults, even though baseline antioxidant enzyme activities of the neonate tend to be lower (6, 7). Recent studies in rabbits indicate that, compared with animals born at term, preterms demonstrate both lower O_2 tolerance and a diminished ability to respond to hyperoxia with increases in antioxidant enzyme activities (8).

Activities of the antioxidant enzymes, CAT, SOD, and GPX, increase in the last 10 to 15% of gestation (9–12). It has been demonstrated that prenatal administration of dexamethasone causes not only increased maturation of the surfactant system, but also increases in antioxidant enzyme activities in the fetus (13). However, because augmentation of enzyme activities in response to hyperoxia may be necessary for tolerance, the effect of prenatal dexamethasone on postnatal enzyme responses to hyperoxia may be more important than its ability to increase baseline activities in fetal animals.

To our knowledge, there is only one preliminary report of antioxidant enzyme responses to hyperoxia in term neonatal animals after prenatal dexamethasone treatment (14) and no studies in preterm animals. The present study was undertaken to investigate whether there are differences after prenatal dexamethasone treatment in the activities of the pulmonary antioxidant enzymes at birth or after short-term hyperoxic exposure of both preterm and term rats.

MATERIALS AND METHODS

Animals. For studies of term pups, timed-gestation pregnant Sprague-Dawley rats weighing 250 to 300 g were obtained from Sasco Inc. (Houston, TX) at gestational d 16 to 17 and were allowed to spontaneously deliver at term (21.5 d). Pregnant females were given injections of dexamethasone sodium phosphate 0.2 mg/kg (Elkins-Sinn, Inc., Cherry Hill, NJ) intramuscularly on d 19 and 20 of gestation. Controls were given injections of an equal volume of 0.9% NaCl (saline). Pups from similar treatment groups were pooled and split between O_2 and air exposure groups.

For preterm studies, pregnant rats were given injections of dexamethasone on d 18 and 19 (24 and 48 h before scheduled preterm delivery). On d 20 (24–36 h before expected term gestation), the females were anesthetized with ketamine 80 mg/kg (Ketalar, Parke Davis, Morris Plains, NJ) and xylazine 4 mg/kg (Gemini TM, Rugby Labs, Inc., Rockville Center, NY). A vertical abdominal incision was made, and the pups were delivered along with the placentas. After delivery of all pups, the umbilical cords were tied with suture and the placentas removed. Pups were washed in warm water and stimulated for a period of 10 to 15 min to establish respiration. They were placed in exposure chambers with a surrogate mother who had spontaneously delivered at term on the same day. For exposures of preterm pups, the entire O_2 exposure cage was placed in an infant incubator (model C86, Air Shields, Inc., Hatboro, PA) with the temperature set to 26 to 27°C.

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Gas exposures. Exposures to either O_2 or air were performed in air-tight plastic cages. O_2 and air were humidified and provided at flow rates of 5.0 L/min. O_2 concentration exiting the cage was analyzed continuously with a Ventronics (Temecula, CA) model 5524 O_2 monitor. O_2 concentrations remained at ≥98% at all times. CO_2 exiting the cage was verified to be <1% using a Fyrite test kit (Bacharach Inc., Pittsburgh, PA).

All preterm pups were exposed to O_2 for 24 h. Term pups were exposed for 24, 48, or 72 h to either O_2 or air. Pups were allowed to nurse *ad libitum* during gas exposures. Dams were allowed free access to water and rat food during exposures, were maintained on a 12-h light-dark cycle, and were switched between O_2 and air exposure groups every 24 h. All experiments were performed with the approval of the Animal Care and Use Committee of the University of Texas Medical Branch.

Preparation of lung homogenates. After anesthesia with sodium pentobarbital, the chest and abdomen were opened, the aorta transected, and the pulmonary artery perfused via the right ventricle with ice-cold PBS, pH 7.4, until the lungs were white. The lungs were then excised from the heart and major bronchi and copiously rinsed with PBS. The lungs were suspended in 5 volumes (wt/vol) of homogenization buffer (50 mM potassium phosphate buffer, 1.0 mM EDTA, pH 7.4) and homogenized on ice for 1 min on setting 7 in a Brinkmann Polytron (Brinkmann Instruments, Inc., Westbury, NY). The homogenate was then sonicated on ice for 40 s after addition of Triton X-100 to a final concentration of 0.005%, centrifuged at 20 000 × *g* for 15 min at 4°C, and the supernatant solution was frozen at −70°C until assayed for enzyme activity.

Biochemical assays. Activities of the antioxidant enzymes were measured as previously reported (15) using standard spectrophotometric methods. GPX activity was measured using the method of Paglia and Valentine (16) (1 unit = nmol NAPH/min), CAT was assayed by the method of Holmes and Masters (17) (1 U = μmol H_2O_2/min), and SOD was assayed using the method of Crapo *et al.* (18). One unit of SOD activity was defined as that amount of activity causing a 50% decrease in the rate of cytochrome *c* reduction/min. DNA was measured spectrophotometrically using the method of Richards (19) with calf thymus DNA as standard. The DNA measurements were repeated due to initial instrument malfunction and therefore were measured after a storage time of longer than 1 mo. Blood contamination of lung homogenates was estimated by measuring Hb concentration using a commercially available kit (Sigma Chemical Co., St. Louis, MO). Blood contamination accounted for 3.26 ± 0.46% of SOD activity, for 5.29 ± 0.81% of CAT, and 0.79 ± 0.08% of GPX activity. These activities are similar to those previously reported (10).

Statistical analysis. Data are expressed as mean ± standard error of enzyme activities. The lungs of three to six neonatal rats were pooled for each lung homogenate measurement. The homogenates from three to seven litters were analyzed in each group. Probabilities of differences between groups were estimated by the Mann-Whitney nonparametric test (20) unless otherwise specified. Differences between groups of $p < 0.05$ were considered to be statistically significant.

RESULTS

Mortality and body growth. In the preterm groups, mortality within the first 30 min of life (early mortality) was attributed to delivery complications with failure of resuscitation. Mortality in the first 30 min of life was higher in the dexamethasone than in the control group ($p = 0.0494$). Thereafter, mortality in the O_2-exposed preterm pups was lower for dexamethasone-treated animals (91%) compared with saline controls (57%) (Table 1). In the saline-treated (control) groups, survival of preterm pups exposed to air was only 4% at 24 h compared with a survival of 57% in O_2. Although survival in the O_2 saline group was 57% after 24 h, it diminished markedly between 48 and 72 h (12% at 72 h). Birth weights of preterms did not differ between the dexamethasone and control groups. Weight loss in preterm pups was observed over the 24-h study period, presumably because of poor nutrition, but the degree of weight loss did not differ between the treatment groups.

The pups born at term had negligible mortality over the 72-h study period regardless of prenatal treatment or type of exposure. Birth weights at term were significantly lower in the dexamethasone group (Table 1), suggesting a steroid effect on prenatal growth. However, weight gains for the term groups at 24 and 72 h did not differ between treatment or exposure groups.

Due to the potential inaccuracy of lung weights after pulmonary artery perfusion, we did not routinely measure weights of individual lungs.

Antioxidant enzyme activities in lung homogenates of preterm pups at birth and after hyperoxic exposure. At birth, the dexamethasone-treated preterm pups had significantly higher CAT and SOD activities than controls (Fig. 1). There were no differences in GPX at birth between the two prenatal treatment groups, although GPX tended to be lower in the dexamethasone group.

In preterms, after 24 h of hyperoxia, activities of the enzymes did not differ between the dexamethasone and control treatment groups. Compared with activities at birth, CAT and SOD increased 23 and 28%, respectively ($p < 0.05$ for SOD), after 24 h of hyperoxic exposure in the control (saline-treated) preterm pups (Fig. 1), whereas there was no change in GPX. Alternatively, in the dexamethasone group, GPX was higher after 24 h of hyperoxia, but there were no changes in SOD and CAT.

Antioxidant enzyme activities in term rat pups at birth and after hyperoxic exposure. In term pups, there were no differences in antioxidant enzyme activities at birth between the dexamethasone and control treatment groups (Fig. 2). In the air-exposed term pups, both CAT and GPX activities rose to a maximum at 48 h, whereas SOD did not change. Although there were trends toward higher activities of SOD and CAT in the dexamethasone-treated group after exposure to air for 24 and 72 h, differences were significant only for SOD at 72 h ($p < 0.05$). After hyperoxic exposure of term pups (Fig. 2), there were no differences in antioxidant enzyme activities between the dexamethasone and control groups.

DISCUSSION

We have examined antioxidant enzyme activities at birth and after hyperoxic exposure in both preterm and term rat pups after

Table 1. *Mortality and body growth in prenatal treatment groups at 0–24 h*

	% Early mortality (<30 min)	% Late mortality (24 h)	Birth weight* (g)	Weight change* (24 h)
Preterm saline	0/48†	13/30 (43%)‡	4.41 ± 0.09	−0.49 ± 0.07
Preterm dexamethasone	5/65 (7.7%)§	4/46 (8.7%)§	4.47 ± 0.31	−0.51 ± 0.19
Term saline	0/85	0/47	6.13 ± 0.15	+1.34 ± 0.21
Term dexamethasone	2/55 (3.6%)	0/38	5.67 ± 0.20§	+0.73 ± 0.24

* Values expressed as mean ± SEM.

† The denominator denotes the number of pups born.

‡ The denominator denotes the number of pups in the 24-h study groups.

§ $p < 0.05$ for saline *vs* dexamethasone within each age group by χ^2 analysis (mortality) and *t* test (weights).

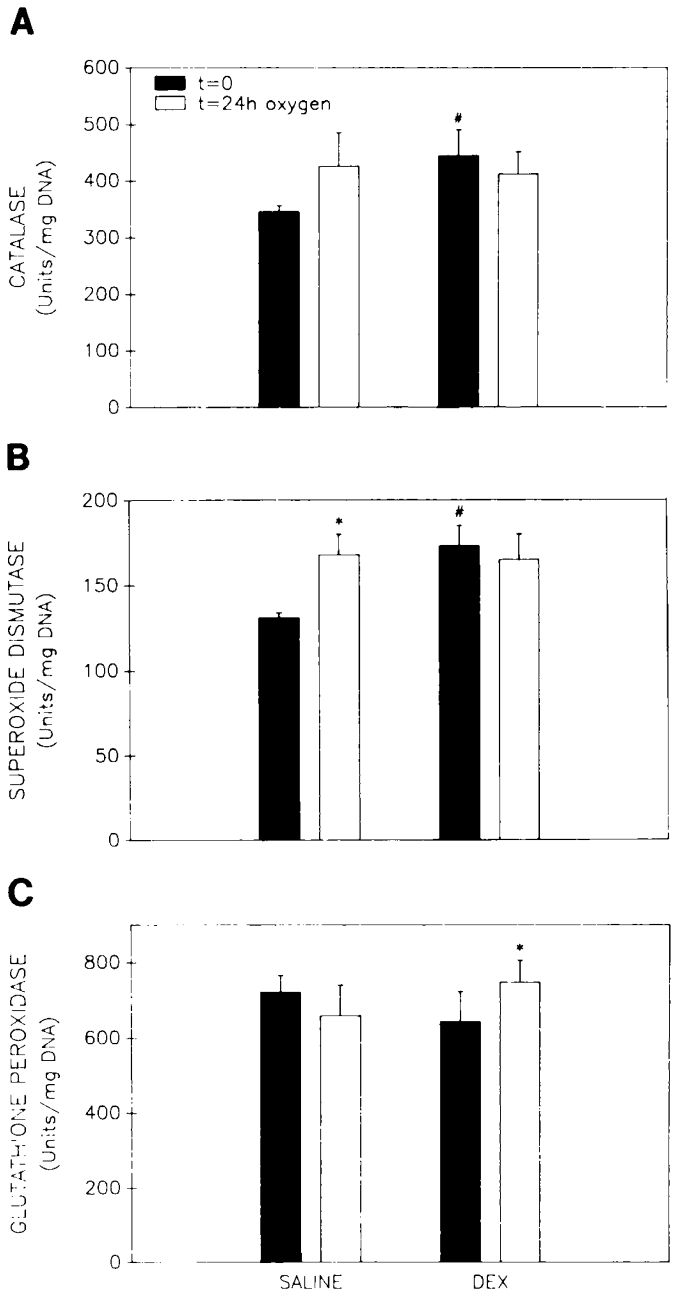

Fig. 1. Comparison of CAT (*A*), SOD (*B*), and GPX (*C*) at birth (t = 0) and after 24 h of >95% O_2 exposure (t = 24) in preterm rat pups treated prenatally with saline or with dexamethasone. Activities are expressed as mean units/mg DNA ± SEM for four separate exposures in each of the groups at birth and in the saline group at 24 h and for seven exposures in the dexamethasone group at 24 h; *, $p < 0.05$ for t = 0 *vs* t = 24 within each prenatal treatment group; #, $p < 0.05$ for saline *vs* dexamethasone treatment.

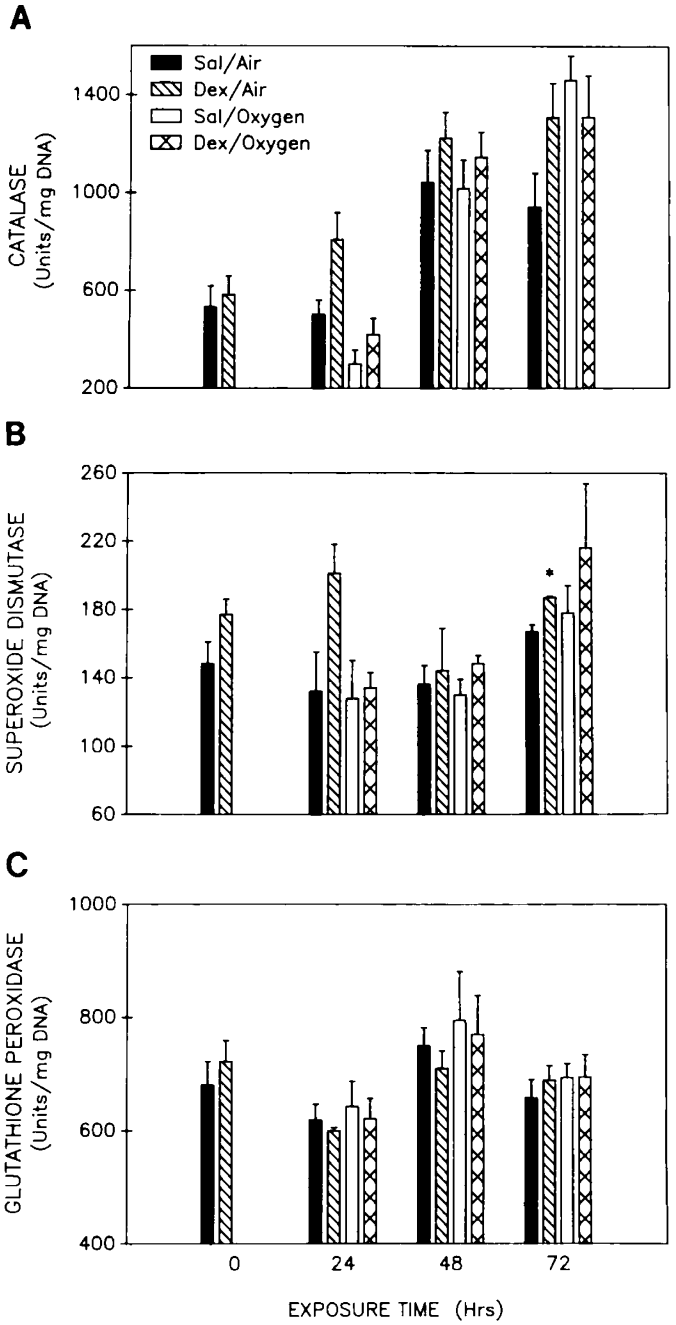

Fig. 2. Activities of CAT (*A*), SOD (*B*), and GPX (*C*) at birth (t = 0) and after 24, 48, and 72 h of exposure to air or >98% O_2 in term rat pups. Activities are expressed as mean units/mg DNA ± SEM for three to six separate exposures. *, $p < 0.05$ for saline/air (*Sal/Air*) *vs* dexamethasone/air (*Dex/Air*). There were no differences in activities between treatment groups after hyperoxic exposure. *Filled bars*, control (saline treatment) at birth and after 24 to 72 h of air exposure; *diagonally hatched bars*, dexamethasone-treated pups at birth and after air exposure; *open bars*, control after O_2 exposure for 24 to 72 h; *cross-hatched bars*, dexamethasone after O_2 exposure.

prenatal dexamethasone treatment. To our knowledge, this is the first report of antioxidant enzyme activities after hyperoxia in the preterm rat or of the early (<7 d) response of term neonatal rats to hyperoxia after prenatal dexamethasone treatment.

A shortcoming of the study was the inability to include a control group of preterm animals exposed to air or a group of preterm animals exposed to hyperoxia for >24 h. Tanswell *et al.* (21) reported similar mortality rates in air-exposed preterm pups, with only a 6% survival at 24 h. Their reported survival of O_2-exposed pups at 36 h was 47%, which was similar to ours (57% at 24 h). These survivals are also similar to those reported by Frank *et al.* (13) of 9% 6-h survival in 40% O_2. However, Tanswell *et al.* (21) reported negligible mortality after 36 h, until the 2nd wk of life. In contrast, our group of preterm rats demonstrated substantial mortality between 48 and 72 h. This may be explained by slight differences in pulmonary maturity. The birth weights of the preterm rats in our control group were less than previously reported birth weights for rats born 24 h prematurely (4.41 *versus* 5.39) (13), suggesting more immaturity. Therefore, the results of study of the saline control group must be interpreted with caution because the survivors may include a group with slightly more pulmonary maturity. However, it is doubtful that it is a group more inherently resistant to O_2 toxicity.

In term neonates at least, O_2-induced mortality begins at about 7 d (6), and Tanswell *et al.* (21) reported that O_2 injury did not occur until the 2nd wk of life in preterm pups.

Our findings of higher SOD and CAT at birth in the dexamethasone preterm groups corroborates a previous report by Frank *et al.* (13). However, the higher activities at birth were not maintained postnatally, and there were no differences between the treatment groups after hyperoxia.

Over the 24-h exposure of the saline control group, there was a significant rise in SOD and a trend toward an increase in CAT, as well. Because we were unable to study a group of air controls, we could not determine whether this was a response to hyperoxia. These postnatal changes may reflect the *in utero* rise in late-gestational enzyme activities previously documented in the rat fetus (9–13). However, because there was a high mortality in this group at 24 h, conclusions are difficult to make. There are no previous studies of postnatal changes in antioxidant enzyme activities in preterm lungs, except in the rabbit. Frank and Sosenko (8) demonstrated no differences in antioxidant enzyme activities after 48 and 72 h of O_2 compared with air exposure in the preterm rabbit, suggesting that preterm rabbits do not respond to hyperoxia with increases in antioxidant enzyme activities. They did not report changes in activities from birth to 48 or 72 h, nor did they study prenatal dexamethasone treatment, so their results cannot be compared with those of the present study.

Of interest is the appearance of a coordinate regulation of CAT and SOD compared with GPX in the preterm groups. Prenatal dexamethasone resulted in higher SOD and CAT at birth, but no change in GPX. After 24 h of hyperoxic exposure, GPX increased in the dexamethasone-treated group, whereas at least SOD increased in the saline group. SOD and CAT are both necessary for complete detoxification of superoxide anion, and coordinate changes in these enzymes would theoretically allow more efficient antioxidant protection.

Our results confirm a previous report that prenatal treatment with dexamethasone does not result in differences in antioxidant enzyme activities at birth in term rats (13). Although there were trends toward higher CAT and SOD activities in dexamethasone-treated term pups after both air and O_2 exposure, these differences did not reach significance except for SOD after 72 h of air exposure. Similarly, preliminary data from Frank (14), who studied term rats after longer durations of O_2 exposure (7–10 d), indicate that prenatal dexamethasone treatment does not result in a later augmentation of enzyme response. Of interest, Frank (14) also reported improved survival under hyperoxic conditions in the dexamethasone-treated term rats. Mechanisms other than the antioxidant enzyme system may be involved in improved survival. Prenatal dexamethasone treatment may result in diminished oxidative stress due to its antiinflammatory effects (22), or it may accelerate maturation of the relatively immature lung structure of the term rat (23–27), thereby improving O_2 tolerance.

In summary, prenatal dexamethasone treatment dramatically improved survival of preterm pups at 24 h. Although prenatal dexamethasone enhances the maturation of the antioxidant enzyme system in late gestation, there were no differences between the treatment groups in enzyme activities after hyperoxia. Although prenatal dexamethasone may protect the term neonate from O_2 toxicity (14), our data suggest that early augmentation of antioxidant enzyme activities in term neonatal rats does not accompany this phenomenon. Further investigation of the effects of prenatal dexamethasone on the lungs of neonatal animals exposed to high O_2 concentrations is indicated.

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