Positive Regulation of Pulmonary Antioxidant Enzyme Gene Expression by Prenatal Thyrotropin Releasing Hormone Plus Dexamethasone Treatment in Premature Rats Exposed to Hyperoxia

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ABSTRACT

Prenatal administration of thyrotropin releasing hormone (TRH) plus dexamethasone (DEX) to pregnant rats produces significantly depressed fetal lung antioxidant enzyme (AOE) activities and AOE mRNA levels in late gestation. Because of this negative regulation of AOE gene expression in the late fetal lung, we hypothesized that hormonally pretreated prematurely delivered rats might demonstrate inferior tolerance to prolonged hyperoxia. Litters of prenatal TRH+DEX-treated and sham-treated prematurely delivered rat pups (gestational d 21 of 22) were randomized to either >95% O₂ or room air for up to 14 d. The right lungs of 2- and 7-d exposure pups were assayed for AOE activities; the left lungs of the same pups were used to quantitate the concentrations of AOE mRNA by solution hybridization. The prenatal TRH+DEX-treated pups were able to induce adaptive lung AOE mRNA and activity responses to hyperoxia by 2 d of exposure; and by 7 d in O₂ they showed greater increases in AOE mRNA concentrations and AOE activities in response to hyperoxic challenge compared with the sham-treated controls. Lung lipid surfactant measurements after hyperoxia were not affected by prenatal TRH+DEX treatment. In addition, TRH+DEX-pretreated premature rats did not show the hypothesized increased susceptibility to O₂-induced lung damage and lethality, but, in fact, had slightly improved hyper-

oxic survival (d 3–7 of O₂ exposure) compared with sham-treated controls. Exposure to hyperoxia significantly reduced serum triiodothyronine and thyroxine levels in the sham-control pups. These findings suggest that although TRH+DEX-pretreated premature rats have decreased lung AOE gene expression at the time of preterm delivery, subsequent hyperoxic exposure is able to convert AOE gene expression to positive regulation which results in more rapid and greater increases in protective AOE activity levels. This regulation is likely mediated differently for the individual AOE. (*Pediatr Res* 37: 611–616, 1995)

Abbreviations

TRH, thyrotropin releasing hormone
DEX, dexamethasone
AOE, antioxidant enzyme
CuZnSOD, copper-zinc superoxide dismutase
MnSOD, manganese superoxide dismutase
CAT, catalase
GP, glutathione peroxidase
DSPC, disaturated phosphatidylcholine
T₃, triiodothyronine
T₄, thyroxine

Prenatal hormonal therapy with maternal administration of a combination of TRH and glucocorticoids is now being extensively tested clinically in threatened premature deliveries to try to rapidly stimulate lung maturation and reduce the incidence and severity of respiratory distress syndrome in premature

infants (1–3). It has been previously demonstrated by our laboratories that although prenatal administration of TRH+DEX to pregnant rats accelerates surfactant system development in the late fetal lung, this same hormonal treatment significantly decreases the normal fetal lung AOE (SOD, CAT, and GP) activity elevations in late gestation (4). This hormonal effect appears to depend on a pretranslational/transcriptional mechanism involving decreased AOE mRNA levels (5). When full-term newborn rats prenatally treated with TRH+DEX were exposed to prolonged hyperoxia, results showed that although these newborns manifested lower baseline AOE activities at birth than control term newborns, the

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hormone-treated pups were still able to induce an adaptive lung AOE response to hyperoxic exposure of equal or even greater magnitude compared with O₂-exposed control offspring (4). Moreover, what was most interesting and still unexplained in this previous study was the consistent finding that, despite their greater pulmonary AOE response to hyperoxic challenge, the prenatal TRH+DEX-treated full-term newborn rats showed evidence of decreased hyperoxic survival and O₂ tolerance compared with O₂ control pups (6).

Based on these previous findings, we hypothesized that prematurely delivered rat pups prenatally treated with TRH+DEX might likewise demonstrate inferior tolerance to prolonged hyperoxia just as full-term TRH+DEX rat pups did. To test this hypothesis, we used a prematurely delivered rat model (7) which we felt was more reflective of the clinical use of this combined hormonal treatment. We performed studies in which prenatal TRH+DEX-treated and sham-treated control premature rat pups were simultaneously exposed to prolonged hyperoxic exposure and various parameters of relative O₂ tolerance/susceptibility were compared between the two groups.

METHODS

Animals and treatment. Adult breeder Sprague-Dawley albino rats were obtained from Charles River Laboratories (Wellington, MA). Breeding was accomplished by placing two female rats in the same cage with one male overnight. Probable pregnancy was determined by a positive vaginal sperm smear the next morning, and the onset of gestation was considered to be the midpoint of the cohabitation period. The timed-pregnancy rats were maintained on standard laboratory food and water *ad libitum* and were kept on a 12-h light/dark cycle in the accredited University of Miami Animal Care Facilities. All portions of the experimental protocol were preapproved by the University of Miami Animal Research Welfare Committee.

At 48 h before premature delivery at gestational d 21, timed-pregnancy rats were divided at random into hormonal treatment or sham-control groups. For the group receiving prenatal TRH+DEX combination therapy, prenatal TRH (Bachem Inc., Torrance, CA) was administered as a loading dose of TRH (25 µg/kg, s.c.) at 48 h before premature delivery, immediately after the s.c. implantation of an Alzet osmotic minipump (Alza Corp., Palo Alto, CA) through which continuous TRH was administered (100 µg/kg/d). In addition, these same dams received prenatal DEX (Steris Laboratories, Inc., Phoenix, AZ) at a dose of 0.4 mg/kg intraperitoneally at 48 and 24 h before premature delivery. The control pregnant rat group received an equivolume s.c. injection of saline instead of TRH, and a "sham" operation using the same ketamine/xylazine anesthesia (ketamine:xylazine, 45 mg/kg:5 mg/kg, intraperitoneal injection), as well as an equivolume (0.5 mL/100 g) of intraperitoneal saline instead of DEX. All of the above hormonal treatment procedures and dosages of TRH and DEX were identical to those used in previously reported studies from our laboratories (4–6).

At 21 d of gestation (term = 22 d), pregnant rats were anesthetized by intraperitoneal injection of ketamine/xylazine

(dose as above) and hysterotomy was performed. The newly delivered pups were rapidly resuscitated by removing the surrounding membranes, drying them vigorously, warming them on heating pads, and applying low flow O₂ (~30%) for ~30 min.

Hyperoxic exposure. Shortly after delivery and stabilization, all the surviving 21-d premature pups of three to five equivalently treated litters were first pooled together and then randomly redistributed to surrogate rat dams that had recently delivered at term. For each group (TRH+DEX or sham-treated control), one half of the reconstituted litters were placed in >95% O₂ exposure chambers and one half remained in room air. Some nonexperimental full-term newborn rat pups (marked by tail snip) were added at 24 h and/or 48 h of exposure when necessary to readjust the litter sizes to 10–12 pups per dam to ensure equal litter sizes for equivalent nutrition. The details of the procedures and conditions of hyperoxic exposures have been previously described (7). Survival was noted daily during the 14-d exposure period.

Biochemical analyses. Randomly selected O₂-exposed and air-exposed pups from each group were killed by an intraperitoneal overdose of pentobarbital after either 2 or 7 d of exposure. The procedures of lung perfusion have been previously described (7). The left lungs of two pups were pooled for measuring AOE mRNA concentrations; the right lungs of the same pups were pooled for assaying AOE activities.

AOE mRNA quantitation. The rat complementary RNA for CuZnSOD, MnSOD, CAT, and GP were prepared as previously described (5, 8, 9). Total nucleic acids were extracted by phenol-chloroform extraction of the left lung tissue homogenate (9). Quantitation of each AOE mRNA was achieved using the solution hybridization assay of Durnam and Palmiter (10). Results of the AOE mRNA concentrations were expressed as the specific AOE mRNA molecules per mg DNA.

AOE activity assays. The right lung tissues were homogenized in cold 2.5 mM potassium phosphate buffer (pH 7.8) in an Omni homogenizer (Omni International Inc., Waterbury, CT). The homogenates were centrifuged at 27 000 × *g* for 45 min at 4°C. The supernatant fractions were then dialyzed overnight against three changes of 50 volumes of 2.5 mM potassium phosphate buffer (pH 7.8) with 0.1 mM EDTA and were subsequently used for analyses of AOE activities using standard spectrophotometric assays for SOD (cytochrome *c* reduction rate by xanthine-xanthine oxidase) (11), CAT (H₂O₂ consumption rate) (12), and GP (NADPH oxidation rate with cumene hydroperoxide substrate) (13). The method of differential cyanide inhibition was used to quantitate cytosolic CuZnSOD and mitochondrial MnSOD activities (14). All enzyme activities were expressed as activity units per mg DNA. DNA in aliquots of the lung homogenates used for AOE mRNA or for AOE activity was extracted and measured using purified calf thymus DNA (Sigma Chemical Co., St. Louis, MO) as a standard (15).

Surfactant phospholipids and DSPC. The lipids were extracted from lung homogenates using the method of Bligh and Dyer (16). A portion of the lipid extracts was used to isolate DSPC according to Mason *et al.* (17). The total phospholipids and DSPC content were measured by phosphorus determina-

tions in these fraction (18). A known quantity of ¹⁴C-dipalmitoyl-phosphatidylcholine (DuPont NEN, Boston, MA) was added before lipid extraction to estimate and correct for sequential losses. Lipids were expressed as mg/mg protein. Protein was measured by the Folin phenol reagent method (19).

Lung pathologic analyses. Randomly selected animals from each group at 7 and 14 d of exposure were killed as above. Unperfused, uninflated left lung lobes were tied off and removed for assessing lung wet weight/dry weight ratios [80°C oven; lungs dried until constant weight found (72 h)]. For microscopic studies, the right lungs of randomly selected animals at 7 d of exposure were fixed *in situ* via a tracheal cannula at a constant inflation pressure of 20 cm H₂O (fixative, 10% buffered formalin). The coded hematoxylin and eosin-stained lung sections (right upper, middle, and lower lobes) were examined for pathologic changes by two investigators who were looking for evidence of interstitial, perivascular/peribronchiolar, and intraalveolar edema, and lung hemorrhage.

Serum hormone assay. Hormone assays for serum T₃ and T₄ were done using specific RIA kits (Incstar Corporation, Stillwater, MN) in TRH+DEX-treated and sham-treated control pups after 14 d of exposure.

Statistical analyses. Comparative survival data and incidence of intraalveolar edema were compared by χ^2 testing. Four group comparisons (TRH+DEX air, TRH+DEX O₂, sham-treated control air, and sham-treated control O₂) were made by analysis of variance and Duncan's multiple range test. Kramer's extension of Duncan's test was used in cases of unequal number of replications (20). For all statistical tests, a $p < 0.05$ value was considered a significant difference between the compared values.

RESULTS

Survival in hyperoxia. Initial survival at ~30 min after premature delivery averaged 77% (330/431, number alive/total number delivered) for sham-treated control rat pups and 78% (272/349) for TRH+DEX-treated rat pups. The composite survival rates of TRH+DEX-treated and sham-treated control premature rat pups during 14 d of hyperoxia are illustrated in Figure 1. Premature rats pretreated with TRH+DEX did not demonstrate the hypothesized inferior tolerance to hyperoxic challenge but actually had slightly improved O₂ survival *versus* sham-treated control rats between exposure d 3 and 7.

Lung biochemical responses to hyperoxia. The comparative responses of the pulmonary AOE to hyperoxic challenge in the TRH+DEX-treated and sham-treated control premature rat pups are shown in Tables 1 and 2 (at 2 d exposure) and Figures 2 and 3 (at 7 d exposure). By 2 d in hyperoxia, the prenatal TRH+DEX pups were able to elevate adaptive lung AOE mRNA levels (Table 1) and increase their CAT and GP activity response to hyperoxic exposure compared with no significant changes in CAT activity and slightly decreased GP activity in the sham-treated control-O₂ pups (Table 2). After 7 d in hyperoxia, the four AOE mRNA levels (Fig. 2) significantly increased in the TRH+DEX group compared with its respective air group and even greater increases in AOE mRNA levels were present in the TRH+DEX O₂ group when compared with the sham-treated control-O₂ group (with the single exception of MnSOD mRNA). The four AOE activities were also consistently greater in the TRH+DEX group than the sham-treated control O₂ pups (Fig. 3).

In contrast to the lung AOE data, no significant differences in response to 7 d of hyperoxia were found in lung DSPC content between the TRH+DEX (0.062 ± 0.007) and sham-

Figure 1. Survival of TRH+DEX-pretreated and control premature rat pups in >95% O₂ for 14 d (n/n , number alive/number put in O₂). *, $p < 0.005$ and **, $p < 0.05$ for the two O₂-exposed group survival rate comparisons. For the premature rat pups maintained in air, the subsequent survival rates for the TRH+DEX-treated group were 83% (at 24 h), 75% (at 48 h), and 75% (2–14 d of exposure). [$p < 0.001$ compared with survival rates of 57% (at 24 h), 52% (at 48 h), and 52% (2 to 14 d of exposure), respectively, for the control pups.] Note: The initial drop in survival during the first 48 h—comparable for both groups—is likely not due to pulmonary O₂ toxicity.

Table 1. Comparative lung AOE mRNA concentrations at 2 d of hyperoxic exposure

Group	AOE mRNA (molecules/mg DNA $\times 10^{-12}$)			
	CuZnSOD	MnSOD	CAT	GP
Control-air	28.4 $\pm$ 1.2	3.57 $\pm$ 0.29	15.1 $\pm$ 0.9	16.1 $\pm$ 2.0
Control-O ₂	38.3 $\pm$ 3.5*	3.51 $\pm$ 0.31	13.1 $\pm$ 0.8	9.9 $\pm$ 1.2*
	($\uparrow$ 35%)	($\downarrow$ 2%)	($\downarrow$ 14%)	($\downarrow$ 38%)
TRH+DEX-air	20.9 $\pm$ 2.7	3.11 $\pm$ 0.21	9.8 $\pm$ 0.7	11.0 $\pm$ 2.0
TRH+DEX-O ₂	42.2 $\pm$ 5.8*	4.13 $\pm$ 0.30	14.0 $\pm$ 1.5*	13.6 $\pm$ 2.0
	($\uparrow$ 102%)	($\uparrow$ 32%)	($\uparrow$ 42%)	($\uparrow$ 23%)

Values are mean $\pm$ SEM for four to six samples/group using rat left lungs.

* $p < 0.05$ vs respective air group.

Table 2. Comparative pulmonary AOE activities at 2 d of hyperoxic exposure

Group	AOE activity (unit/mg DNA)			
	CuZnSOD	MnSOD	CAT	GP
Control-air	19.3 $\pm$ 0.4	3.25 $\pm$ 0.20	63.7 $\pm$ 4.7	0.22 $\pm$ 0.01
Control-O ₂	20.7 $\pm$ 0.7	3.27 $\pm$ 0.19	71.3 $\pm$ 3.7	0.18 $\pm$ 0.01
	($\uparrow$ 9%)	($\uparrow$ 1%)	($\uparrow$ 12%)	($\downarrow$ 15%)
TRH+DEX-air	19.4 $\pm$ 0.4	2.91 $\pm$ 0.25	61.9 $\pm$ 2.9	0.20 $\pm$ 0.01
TRH+DEX-O ₂	20.9 $\pm$ 0.7	3.36 $\pm$ 0.24	82.2 $\pm$ 1.9*	0.23 $\pm$ 0.01†
	($\uparrow$ 8%)	($\uparrow$ 15%)	($\uparrow$ 33%)	($\uparrow$ 17%)

Values are mean $\pm$ SEM for four to six samples/group using rat right lungs from same samples as Table 1.

* $p < 0.01$ vs TRH+DEX-air group.

† $p < 0.05$ vs control-O₂ group.

treated control pups (0.058 ± 0.005). (Average air DSPC value = 0.051 ± 0.003 mg/mg protein; mean $\pm$ SEM, $n = 8$ samples/group, $p > 0.05$.) There were also no differences with 7 d of hyperoxia in lung total phospholipid content between the TRH+DEX (0.284 ± 0.019) and sham-treated control pups (0.263 ± 0.013). (Average air control total phospholipid value = 0.274 ± 0.008 mg/mg protein; $p > 0.05$.)

Lung pathologic analyses. The comparative lung wet weight/dry weight ratios (a reflection of pulmonary edema)

after 7 and 14 d of hyperoxia for the TRH+DEX and sham-treated control groups are summarized in Table 3. Whereas no differences were present between groups after 7 d hyperoxic exposure, after 14 d of hyperoxia, TRH+DEX-treated preterm pups demonstrated a significantly smaller increase in lung wet/dry weight ratio compared with sham-treated control preterm pups, suggesting less pulmonary edema. As more definitive evidence of advanced O₂ toxicity, light microscopic examination showed a similar incidence of intraalveolar edema in the two O₂ groups after 7 d of exposure. The number with intraalveolar edema fluid per total number of coded lung sections examined was 3/30 (10%) for the TRH+DEX-treated O₂ group, and 2/15 (13%) for the sham-treated control O₂ group ($p > 0.05$). The TRH+DEX-treated premature pups did not show the hypothesized increase in susceptibility to O₂-induced lung damage.

Hormone assays. The T₃ and T₄ serum levels in TRH+DEX-treated and sham-treated control offspring after 14 d in $>95\%$ O₂ or room air exposure are shown in Table 4. Prenatal TRH+DEX treatment resulted in consistently increased T₃ and T₄ serum levels. Exposure to 14 d of high O₂ significantly reduced serum T₃ and T₄ levels in sham-treated control pups and reduced the serum T₄ level in the prenatal TRH+DEX-treated group.

DISCUSSION

The effects of prenatal hormonal treatment with the combination of TRH plus glucocorticoids on the maturation of the fetal lung and the surfactant system have been tested in both extensive animal studies and recent clinical studies. The clinical trials with combined TRH and glucocorticoid treatment have shown a decreased incidence of chronic lung disease (bronchopulmonary dysplasia) in very-low-birth weight infants, but surprisingly no significant decrease in the incidence or severity of respiratory distress syndrome (2, 3).

Figure 2. Comparative lung AOE mRNA concentrations of TRH+DEX-pretreated and control premature rat pups at 7 d of hyperoxic ($>95\%$ O₂) exposure. Rat left lungs were assayed for CuZnSOD mRNA, MnSOD mRNA, CAT mRNA, and GP mRNA by solution hybridization using respective ³⁵S-labeled complementary RNA probes. Values are based on six lung samples/group; bars indicate mean $\pm$ SEM. *, $p < 0.05$ for O₂ group vs respective air group. †, $p < 0.01$ for TRH+DEX O₂ group vs control-O₂ group values.

Figure 3. Comparative pulmonary AOE activities of TRH+DEX-pretreated and control premature rat pups at 7 d of hyperoxic (>95% O₂) exposure vs room air AOE activity levels. Rat right lungs from the same samples as Figure 2 were assayed for CuZnSOD, MnSOD, CAT, and GP activities. Values are based on six lung samples/group; bars indicate mean $\pm$ SEM. *, $p < 0.01$ for O₂ group vs respective air group. †, $p < 0.01$ for TRH+DEX O₂ group vs control-O₂ group values.

Table 3. Comparative lung wet/dry weight ratio after hyperoxic exposure

Exposure	7 d	14 d
Control-air	5.17 $\pm$ 0.07	4.92 $\pm$ 0.03
Control-O ₂	5.24 $\pm$ 0.14 ($\uparrow$ 1%)	6.76 $\pm$ 0.26* ($\uparrow$ 37%)
TRH+DEX-air	5.29 $\pm$ 0.09	5.03 $\pm$ 0.03
TRH+DEX-O ₂	5.47 $\pm$ 0.12 ($\uparrow$ 3%)	6.10 $\pm$ 0.10*† ($\uparrow$ 21%)

Values are mean $\pm$ SEM for five to 11 samples/group for 7 d of exposure and 12 to 15 samples/group for 14 d of exposure.

* $p < 0.01$ vs respective air group.

† $p < 0.01$ vs control-O₂ group.

Table 4. Serum T₃ and T₄ levels in TRH+DEX-treated and control offspring after 14 d in >95% O₂ or room air

	T ₃ (nmol/L)	T ₄ (nmol/L)
Control-air	0.66 $\pm$ 0.09	48.4 $\pm$ 2.3
Control-O ₂	0.32 $\pm$ 0.10*	29.7 $\pm$ 4.0*
TRH+DEX-air	1.16 $\pm$ 0.11†	60.0 $\pm$ 3.7†
TRH+DEX-O ₂	1.24 $\pm$ 0.17†	42.6 $\pm$ 4.7*†

Values are mean $\pm$ SEM for seven to 13 samples/group.

* $p < 0.05$ vs respective air group.

† $p < 0.05$ for TRH+DEX group vs respective control-air or control-O₂.

The premature rat model using 21-d gestation rat pups (7) in the present study provides an appropriate model for investigating prenatal TRH and glucocorticoid treatment (relevant to the human clinical prenatal use), especially because the previously reported full-term animal data (6) strongly imply negative effects on hyperoxic tolerance associated with prenatal TRH+DEX treatment. Our original hypothesis in the present study was that premature rat pups prenatally treated with TRH+DEX would demonstrate inferior tolerance to prolonged hyperoxia, similar to what had been found for full-term TRH+DEX-treated rat pups. Unexpectedly, we found that TRH+DEX-pretreated prematurely delivered pups (unlike hormonally treated term newborns) had transiently improved

hyperoxic survival and no greater sensitivity to pulmonary O₂ toxicity than the sham-treated control pups. These discordant results suggest different effects of prenatal hormonal treatment on preterm *versus* full-term rat pups, because all hormonal treatment procedures and dosages of TRH and DEX were identical for both studies except for the 24-h difference in the timing of the gestational administration of prenatal TRH+DEX.

The molecular biology studies described herein were undertaken to provide insight into the regulatory mechanism by which hormonal intervention (TRH+DEX) might be controlling the normal gene expression of the protective AOE system under subsequent hyperoxic conditions. Our findings suggest that the regulation of lung AOE gene expression is complex under hyperoxic conditions. The prematurely delivered rats pretreated with TRH+DEX were previously found to have lowered lung AOE gene expression (depressed AOE activities and AOE mRNA levels) at preterm delivery at d 21 (4, 5). Similarly, when examined at 2 d postnatal age after room air exposure, their pulmonary AOE mRNA levels remained lower in the premature TRH+DEX group compared with the sham-treated group. However, hyperoxic exposure of premature TRH+DEX-treated pups was able to switch AOE gene expression from negative regulation to positive regulation and result in more rapid and even greater increases in protective AOE activity levels than in sham-treated control premature pups exposed to high O₂.

Previous studies from our own laboratories have demonstrated that normal perinatal gene expression of the four AOE is not coordinately regulated (8). Other studies suggest this is true also under hyperoxic conditions. For example, differential regulation of specific AOE in response to oxidants (21) or to hyperoxia (22) *in vitro* was reported recently in cultured human cells and in hamster tracheal epithelial cells. A recent detailed study by Clerch and Massaro (23) of neonatal rats exposed to hyperoxia has also suggested that lung AOE gene expression is

differentially regulated in high O_2 . Our data suggest that AOE gene expression does not appear to be coordinately regulated in the premature rat lung under hyperoxic conditions and/or plus hormonal intervention. For example, in the sham-treated premature rat, lung CAT mRNA concentration decreased 11% with hyperoxic exposure for 7 d, whereas CAT activity increased 56%, suggesting a translational/posttranslational mechanism of CAT gene expression in the premature rat lung exposed to hyperoxia. The very similar GP activity (102%) and mRNA (108%) responses to hyperoxia (7 d), however, point to regulation of GP in hyperoxia at a pretranslational level. The effects of prenatal TRH+DEX administration on the premature lung AOE responses to hyperoxia seem to indicate activation of other means of regulation. For example, for the TRH+DEX group, we found that the CAT response to hyperoxia consists of an mRNA increase of 20% and activity increase of 72%, which may represent a combined pretranslational and a translational/posttranslational mechanism of gene expression in this group's response to hyperoxia. This appears to be the case for GP as well in the TRH+DEX group exposed to hyperoxia (*i.e.*, more complex AOE gene expression responses than in the sham control group). However, more definitive conclusions about the mechanism(s) of AOE gene expression still depend on further studies to determine whether or not comparative AOE mRNA stabilities (half-lives) are also altered under hyperoxic conditions in either or both the sham control and TRH+DEX-treated rat pup lungs.

More needs to be said about the effect of thyroid hormones in influencing sensitivity to O_2 . The findings in the present study that exposure to high O_2 significantly reduced serum T_3 and T_4 levels in sham-treated control rats (and even reduced serum T_4 level in the prenatal TRH+DEX-treated O_2 group) are in agreement with the study of Galton (24), which demonstrated a marked fall in the serum concentration of endogenous T_4 and a decrease in T_4 -binding activity in serum with hyperoxia. Smith *et al.* (25) previously reported that feeding desiccated thyroid or injecting T_4 to young rats increased the noxious effects of high O_2 exposure. Smith's results are consistent with the previous full-term newborn rat studies from our laboratories in which prenatal TRH administration produced decreased hyperoxic survival and O_2 tolerance associated with increased serum T_3 and T_4 levels (6). For premature rat pups in the present study, however, the similar serum T_3 and T_4 increases in prenatal TRH (plus DEX)-treated rats were not associated with inferior tolerance to hyperoxic exposure. These discordant results for the influence of thyroid hormones on O_2 sensitivity, as well as the divergent effects of prenatal hormonal treatment on preterm *versus* full-term newborn rats would seem to be gestationally related.

Finally, our findings of a positive correlation between the ability to augment AOE gene expression and improved lung tolerance to hyperoxic challenge in our hormonally treated premature animals may further support the continued clinical

use of prenatal TRH and glucocorticoid treatment in threatened premature deliveries with a high likelihood of requiring assisted ventilation with high O_2 therapy. In addition, our data may contribute some understanding of why to date this treatment regimen has had its most pronounced clinical effect on reducing the incidence of bronchopulmonary dysplasia (rather than respiratory distress syndrome).

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