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Prenatal Hormone Treatment with Thyrotropin Releasing Hormone and with Thyrotropin Releasing Hormone Plus Dexamethasone Delays Antioxidant Enzyme Maturation but Does Not Inhibit a Protective Antioxidant Enzyme Response to Hyperoxia in Newborn Rat Lung

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ABSTRACT. Whereas glucocorticoid administration to pregnant rats produces parallel acceleration of lung surfactant and antioxidant enzyme system maturation in late gestation, prenatal thyroid hormone treatment results in acceleration of surfactant maturation, with a paradoxical decrease in antioxidant enzyme (AOE) development. In these studies, we tested whether prenatal thyroid releasing hormone (TRH) treatment would act like prenatal thyroid hormone on pulmonary surfactant and AOE system maturation and whether combined prenatal treatment with TRH plus dexamethasone (DEX) would alter these effects. Secondly, we tested whether prenatal TRH and prenatal TRH plus DEX would inhibit the ability of newborn rats to respond to hyperoxia with protective increases in AOE activities. Results of the developmental studies revealed significantly increased fetal lung disaturated phosphatidylcholine content with significantly decreased pulmonary AOE activities as a result of prenatal TRH treatment that was not reversed with the addition of DEX. Combined TRH plus DEX treatment resulted in statistically significant decreases in body weight, lung weight, and lung weight to body weight ratios at both 21 and 22 d of gestation; growth effects were not seen with TRH alone. In terms of hyperoxic AOE response, despite being born with lower baseline AOE levels, the newborn animals prenatally treated with TRH or TRH plus DEX were able to induce a normal pulmonary AOE response to high O_2 exposure. Although requiring further investigation, this reassuring finding suggests that clinical prenatal therapy with TRH or the combination of TRH plus DEX is not contraindicated for those infants delivered prematurely who go on to require intensive hyperoxic therapy. (*Pediatr Res* 30: 522–527, 1991)

Abbreviations

TRH, thyrotropin releasing hormone
T_3, 3,3′,5-triiodo-L-thyronine
T_4, thyroxine
DEX, dexamethasone
AOE, antioxidant enzyme
SOD, superoxide dismutase
CAT, catalase
GP, glutathione peroxidase
DSPC, disaturated phosphatidylcholine
TPL, total phospholipid

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At birth, the newborn lung is exposed to several-fold higher oxygen tension than during its intrauterine development. To prepare for this transition from the *in utero* to the extrauterine milieu and for its assumption of respiratory function, the developing lung increases both its surfactant content and its AOE levels during the final 10 to 15% of gestation (1). The maturation of both of these lung biochemical systems may be crucial in the neonatal adaptation to independent respiration. Cell survival requires that the cell has adequate constitutive antioxidant defense mechanisms and that it has the capacity to rapidly respond to oxidant stress by an increase in the activity of those defense systems that can detoxify reactive species of O_2 and thereby prevent O_2 toxicity. CAT, SOD, and the enzymes of the glutathione redox cycle (GP, glutathione reductase, and glucose 6-phosphate dehydrogenase) are the primary enzymatic intracellular antioxidant defense mechanisms that function together to detoxify the cytotoxic species—superoxide radical, H_2O_2, and lipid hydroperoxides (2).

It has been demonstrated recently that in at least five species—the rat, rabbit, guinea pig, hamster, and sheep—the development of the surfactant system and the pulmonary AOE system share a chronologically similar late gestational pattern of development (3–6). Experimental evidence also suggests that these two biochemical systems, in addition to their parallel developmental patterns, may share some of the same hormonal regulators as well. We have previously demonstrated that the administration of DEX to pregnant rats in late gestation produced acceleration in both surfactant and AOE system development in their fetal offspring (7). However, whereas the administration of T_3 to pregnant rats in late gestation produced acceleration in fetal lung surfactant development, the T_3 effect on the AOE system development was opposite to that seen for surfactant, with fetal offspring demonstrating delayed pulmonary AOE system development (8).

Thyroid gland production of T_4 and T_3 is controlled by the pituitary hormone thyroid stimulating hormone. Thyroid stimulating hormone, in turn, is controlled by the hypothalamic tripeptide TRH (L-pyroglutamyl-L-histidyl-L-prolyl amide), which is also a potent prolactin releasing factor (9). TRH is an

attractive effector of lung maturation because it readily passes from the maternal to the fetal blood stream and causes thyroid axis stimulation (10, 11). Prenatal administration of TRH to pregnant rabbits has been shown to result in increased pulmonary surfactant production as judged by fetal lung lavage surfactant content (10). Prenatal TRH has also been shown to improve lung compliance in ventilated preterm rabbits (12). Based on the information available on TRH, we hypothesized that TRH would act like T_3 treatment on the developing surfactant and AOE system in the late gestation fetal rat lung.

To further evaluate the hormonal regulation of these two important lung systems, we undertook a series of experimental studies to examine the effects of prenatal TRH treatment alone and TRH in combination with prenatal DEX treatment. The studies were designed to determine *1*) whether prenatal TRH administration in the rat acts like T_3 to accelerate surfactant maturation but depress the maturation of the AOE system; *2*) whether the combined use of prenatal DEX plus TRH would alter the effects of TRH treatment alone on surfactant and AOE system maturation; and *3*) in addition to the effects on AOE and surfactant system development, whether prenatal treatment with TRH or TRH plus DEX would alter the ability of the newborn offspring to mount a protective AOE response during hyperoxic exposure.

MATERIALS AND METHODS

The study was conducted in two parts. Part 1 addressed the development of the AOE and surfactant systems after prenatal administration of TRH alone or the combined therapy of TRH plus DEX. Part 2 addressed the response of the AOE and surfactant systems to 5 d of hyperoxia after prenatal hormone treatment.

Developmental Studies—Part 1. Animals and treatment. Adult Sprague-Dawley albino female rats (~250 g) were bred by placing male and female animals together overnight, checking for sperm-positive vaginal smears the following morning, and considering the midpoint of the cohabitation period as the onset of pregnancy. The timed-pregnant rats were maintained on standard laboratory food and water *ad libitum* and kept on a 12-h light/dark cycle.

At 48 h before premature delivery at 21 d or full-term delivery at 22 d gestation, rats were divided into control or treatment groups. For studies examining TRH (Bachem Inc., Torrance, CA) treatment alone, prenatal TRH was administered as a loading dose of TRH s.c. (25 μg/kg) at 48 h before delivery and by s.c. implantation of an Alzet osmotic minipump (Alza Corp., Palo Alto, CA) through which continuous TRH was administered (100 μg/kg/d). These doses were chosen based on previous works of Rooney *et al.* (10) and Ikegami *et al.* (12). The control groups received an equivolume s.c. injection of saline, as well as a "sham" operation under similar anesthesia. For the studies involving prenatal TRH plus DEX combination therapy, TRH was administered as described above; prenatal DEX was administered at 48 and 24 h before delivery at a dose of 0.4 mg/kg intraperitoneally. The control groups received an equivolume dose (0.5 mL/100 g) of intraperitoneal saline.

At 48 h after initiation of prenatal hormone treatment, rat fetuses of gestational age 21 or 22 d were delivered by hysterotomy under ketamine:xylazine anesthesia (90 mg/kg:10 mg/kg) (Ketalar, Parke-Davis, Morris Plains, NJ and Rompun, Paynet Division-Cutter Labs, Shawnee, KS). All of the animal treatment procedures were preapproved by the University of Miami Committee on Research Animal Welfare.

Lung Biochemistry. Fetuses or newborn pups were killed by an intraperitoneal injection of sodium pentobarbitol followed by exsanguination by severing the great vessels in the abdomen. Their lungs were perfused immediately *in situ* via the pulmonary artery, using iced saline. The left atrial appendage was cut to facilitate drainage of the perfusate. The perfused lungs were then removed, stripped of nonpulmonary tissue, weighed, and homogenized in 20–30 times their weight in cold saline in a polytron (Brinkmann Instruments Co., Westbury, NY) (high speed, 90 s). Two to four lungs were pooled per sample so that adequate lung tissue would be provided for the assays. No differentiation between male and female fetuses was made.

Aliquots of the lung homogenate were analyzed for AOE activities using standard spectrophotometric assays for SOD, CAT, and GP. The SOD assay measures the rate of reduction of cytochrome C at 550 nm, in the presence of 10^{-5} M cyanide (to inhibit cytochrome oxidase activity). Both cytosolic Cu-Zn SOD and mitochondrial Mn-SOD are measured by this method (13). CAT, present in the cytosol and peroxisomes of cells, was measured by the rate of reduction of H_2O_2 substrate at 240 nm (14). GP activity was assayed spectrophotometrically at 340 nm by following the rate of oxidation of NADPH. The assay mixture for measurement of this cytosolic enzyme includes cumene hydroperoxide as primary substrate, with sodium azide added to inhibit contributing activity from CAT enzyme (15). Purified enzyme standards for these assays were obtained from Sigma Chemical Co. (St. Louis, MO) and from Boehringer-Mannheim Biochemicals (Indianapolis, IN).

Aliquots of the lung homogenate were also analyzed for DNA and protein content (16, 17), using purified standards from Sigma Chemical Co. Results of the AOE assays were expressed as units of enzyme activity per mg of DNA (and although not reported, were also calculated per mg protein and per g lung wet wt).

For phospholipid analysis, lipid extraction of lung homogenate aliquots was performed according to the procedure of Bligh and Dyer (18), and the lipid extract, once dried under nitrogen, was then frozen at −70°C before phospholipid analysis (18). An aliquot of lipid extract was designated for measurement of TPL. A second aliquot was used for quantitating DSPC, using the method of Mason *et al.* (19). After separation of the DSPC from the other phospholipids, both the DSPC sample and the TPL sample were analyzed for inorganic phosphorus as described by Morrison (20). A known quantity of ^{14}C-dipalmitoyl-phosphatidylcholine (New England Nuclear, Boston, MA) was added before lipid extraction, and aliquots were counted at each step to correct for sequential losses. Lipids were expressed as mg per g lung wet wt (and also calculated per mg of protein and as the ratio of mg DSPC to mg TPL).

Serum Hormone Assays. From randomly selected TRH-treated or control dams, hormone assays for serum T_3, T_4, and prolactin were done using specific RIA kits (Cambridge Medical Diagnostic, Billerica, MA). Pooled fetal serum (approximately one litter/sample) from randomly selected TRH-treated or control dams was collected for similar hormonal assays.

Hyperoxia Studies—Part 2. Animals and treatment. At 48 h before spontaneous delivery of full-term offspring, pregnant rats were randomly assigned to either a control (saline) group, TRH treatment group or the combined TRH plus DEX treatment group.

After prenatal hormonal treatment (dosages and treatment regimens as described above for the developmental studies), rat newborns were obtained after normal parturition within 6–12 h of the beginning of delivery of the first pup. The newborn pups of several equivalently treated litters were first mixed and the redistributed to the equivalently treated, newly delivered dams. Dams plus litters (10–12 pups) were then divided into either hyperoxia exposure or air exposure groups. Exposures were conducted in 3.5-cubic foot clear plastic exposure chambers. The O_2 and CO_2 levels in the chamber were monitored continuously with Beckman model OM-11 and LB-2 gas analyzers (Beckman Instruments, Inc., Schiller Park, IL). The O_2 concentration was maintained at 96–98%, and the CO_2 concentration was less than 0.4% throughout the exposure period. The temperature in the chambers was 24–26°C and the humidity was 50–70% (in-chamber thermometers/hygrometers). Animals were fed Ralston-Purina (St. Louis, MO) pellet diet and given water *ad*

libitum. The mothers were interchanged daily between litters exposed to O_2 and room air to avoid O_2 intoxication in the nursing dams. The chambers were opened daily (10–15 min) to check on the survival, to exchange air and O_2 dams, and to provide fresh food, water, and cages. All of the procedures were approved by the University of Miami Committee on Research Animal Welfare.

Lung Biochemistry. After 5 d of hyperoxia or room air exposure, the pups were killed and their lungs perfused, removed, and homogenized as described for part 1. Aliquots of the lung homogenate were analyzed for AOE activities, DNA and protein content, and phospholipid and DSPC analysis as described in part 1.

Statistical Analysis. For each assay, multivariate analysis was used to assess the significance of difference between the control and the treatment groups. A large number of variables were calculated and, therefore, the required level for statistical significance was set at different values depending upon the experiment. Using the Bonferroni multiple comparison method, the level for statistical significance was determined to be $p < 0.006$ for those experiments that required TRH plus DEX and $p < 0.004$ for those experiments that required TRH only (21). The t test was used for the hormone assays, with the level of statistical significance at $p < 0.05$. Statistical consultation was obtained from the University of Miami Biostatistics Department.

RESULTS

Developmental Studies—Part 1. General features of prenatal TRH-treated, TRH plus DEX-treated, and control offspring. Physical characteristics of control and hormone-treated offspring at gestational d 21 and 22 are summarized in Table 1. At 21 and 22 d of gestation, the TRH-treated offspring demonstrated statistically significant decreases in body weight and lung weight, with no significant change in lung weight to body weight ratio. Combined TRH plus DEX treatment resulted in statistically significant decreases in body weight, lung weight, and lung weight to body weight ratios at both 21 and 22 d of gestation.

Serum hormone assays. The T_3, T_4, and prolactin serum levels in TRH-treated *versus* control dams are shown in Table 2. Prenatal TRH treatment resulted in statistically significant increases in T_3 and T_4 serum levels with a significant decrease in prolactin serum levels. (Attempts at hormonal analysis of pooled fetal serum yielded values below the assay limits for T_3, T_4, and prolactin for both hormone-treated and control fetuses.)

Table 1. *Physical characteristics of TRH only, TRH + DEX, and control offspring**

	Body wt (g)	Lung wt (g)	LW/BW (%)
TRH treatment alone			
21 d gestation			
TRH	3.65 ± 0.18†	0.121 ± 0.086†	3.33 ± 0.16
Control	4.01 ± 0.17	0.148 ± 0.019	3.70 ± 0.55
22 d gestation			
TRH	5.76 ± 0.20†	0.121 ± 0.001†	1.93 ± 0.13
Control	6.52 ± 0.63	0.143 ± 0.025	2.18 ± 0.19
TRH + DEX treatment			
21 d gestation			
TRH/DEX	4.01 ± 0.57†	0.106 ± 0.016†	2.30 ± 0.23†
Control	4.99 ± 0.51	0.132 ± 0.015	2.95 ± 0.25
22 d gestation			
TRH/DEX	4.52 ± 0.31†	0.077 ± 0.186†	1.71 ± 0.43†
Control	5.58 ± 0.71	0.108 ± 0.100	2.19 ± 0.49

* Values are means ± 1 SD for six to 13 litters/group/gestational age. LW/BW, lung wt to body wt ratio.

† Statistically significant with a $p < 0.006$ for the litters treated with TRH plus DEX compared with controls (saline-treated) and a $p < 0.004$ for the litters treated with TRH alone compared with control litters.

Table 2. *Serum T_3, T_4, and prolactin levels in TRH-treated and control dams**

	T_3 (nmol/L)	T_4 (nmol/L)	Prolactin (μg/L)
TRH treated	0.849 ± 0.227†	14.80 ± 4.76†	4.52 ± 1.01†
Control	0.410 ± 0.172	10.81 ± 4.50	5.56 ± 0.89

* Values are means ± 1 SD for 15 dams in each group.

† Statistically significant with a $p < 0.05$ for the TRH-treated compared with control dams.

Table 3. *Lung tissue DSPC and TPL content in TRH-treated, TRH + DEX-treated, and control offspring**

	DSPC	TPL
TRH treatment alone		
21 d gestation		
TRH	3.27 ± 0.21†	15.8 ± 1.2
Control	2.76 ± 0.10	15.0 ± 0.9
22 d gestation		
TRH	5.64 ± 1.29†	24.5 ± 8.8†
Control	3.62 ± 0.82	16.9 ± 3.4
TRH + DEX treatment		
21 d gestation		
TRH/DEX	4.49 ± 1.36†	14.8 ± 4.9
Control	3.42 ± 0.85	15.3 ± 4.9
22 d gestation		
TRH/DEX	5.16 ± 1.19†	23.0 ± 6.1
Control	4.02 ± 0.93	22.0 ± 5.9

* Values are means ± 1 SD for six to 13 litters/group/gestational age. DSPC and TPL are expressed as mg/g lung.

† Statistically significant with a $p < 0.006$ for the litters treated with TRH + DEX and a $p < 0.004$ for the litters treated with TRH alone compared with control litters.

Lung biochemistry. The developmental pattern of lung tissue surfactant (DSPC) and TPL content (expressed as mg/g lung) in TRH-treated and TRH plus DEX-treated *versus* control offspring at gestational d 21 and 22 are shown in Table 3. Offspring of TRH-treated dams had statistically significant increases in lung tissue DSPC content compared with control values at 21 and 22 d of gestation. Similarly, offspring of TRH plus DEX-treated dams had significantly increased mean lung tissue DSPC content compared with controls at both gestational days studied (The same relationship held true when DSPC and TPL content were expressed per mg of protein rather than per g wet wt of the lung). There was no effect on protein, DNA, or protein/DNA ratios in the hormonally treated offspring.

The comparative late gestational development of the pulmonary AOE system in the three experimental groups is illustrated in Figure 1. At 21 d of gestation, the TRH-treated offspring demonstrated statistically significant decreases in pulmonary SOD (40%), CAT (20%), and GP (35%) enzyme activities compared with control group values, and at 22 d gestation had statistically significant decreases in SOD (47%) and CAT (18%) activities with a decrease in GP (16%) activity that approached significance when compared with controls. The addition of DEX treatment to prenatal TRH treatment did not reverse the inhibitory effect of TRH on lung AOE system development, but instead produced significantly depressed levels at gestational d 21 and 22 for each AOE studied, with CAT (38% decrease d 22) and GP (41% decrease d 22) levels even further depressed than with TRH treatment alone.

Hyperoxia Studies—Part 2. Lung biochemistry. After hyperoxic exposure, TRH-treated offspring showed a statistically significant increase in the lung tissue surfactant (DSPC) and TPL content (expressed as mg/g lung), which increased 24 and 21%, respectively (*versus* increases of 18 and 6%, respectively, in controls). In TRH plus DEX offspring, after hypoxic exposure there were statistically significant increases in DSPC and TPL

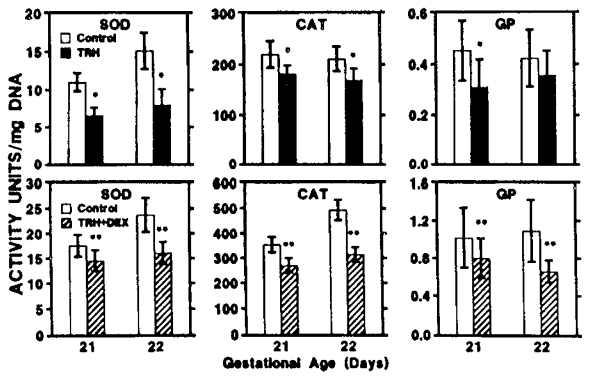

Fig. 1. Effect of prenatal hormonal treatment on lung AOE system development. Values are mean ± 1 SD for six to 13 litters per group per gestational age. AOE activity levels are calculated per mg DNA. *, Statistically significant with a $p < 0.004$ for the litters treated with TRH alone compared with controls (saline-treated); **, statistically significant with a $p < 0.006$ for the litters treated with TRH plus DEX compared with control litters.

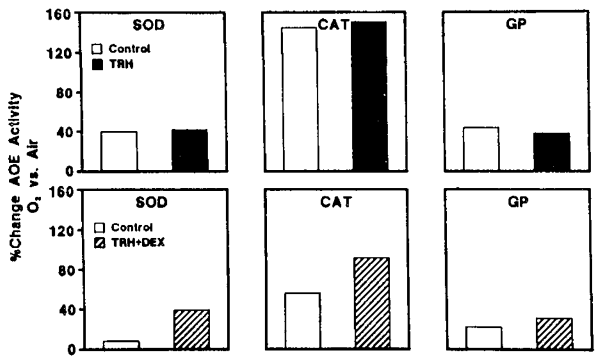

Fig. 2. Comparative AOE responses to hyperoxia (>95% O_2 for 5 d) in hormonally treated and control offspring. Values are % change in activity for AOE in O_2 exposed rat lungs compared with air-exposed lung AOE values (n = eight to 12 litters per group). AOE activity levels of air-controls for TRH experiments: SOD 9.07 ± 3.79; CAT 294 ± 45; and GP 0.23 ± 0.63 units/mg DNA. AOE activity levels of air control for TRH + DEX experiments: SOD 6.31 ± 2.43; CAT 181 ± 36; and GP 0.27 ± 0.02 units/mg DNA. All O_2 values are significantly increased compared with respective air controls. Percentage of change in AOE activities are similar in hormonally treated *vs* control pups in O_2.

content, which increased 44 and 36%, respectively (*versus* increases of 13 and 11%, respectively, in controls). The same relationships held true when DSPC and TPL were expressed per mg of protein rather than per g wet wt of lung.

The activity changes of the pulmonary AOE after 5 d of hyperoxia (expressed as % change compared with room air-exposed activity levels) are shown in Figure 2. Despite lower AOE levels at the time of birth (Fig. 1), the newborn animals prenatally treated with either TRH alone or TRH plus DEX were able to induce an adaptive AOE response to hyperoxic exposure of similar or even greater magnitude compared with control O_2-exposed offspring.

DISCUSSION

The late gestational time course of maturation of the surfactant system and the pulmonary AOE system is similar in all tested animal species (22). The development of these two critical lung biochemical systems has been shown to be not only chronologically related, but also related by some similar hormonal control mechanisms; *i.e.* prenatal DEX therapy has been found to accelerate both fetal lung surfactant system and AOE system maturation, whereas prenatal metyrapone (an inhibitor of endogenous glucocorticoid synthesis) treatment causes significantly delayed development of both lung systems (23). In terms of thyroid hormones, however, unlike their role in promoting surfactant system maturation (24–29), prenatal T_3 treatment has been found to significantly depress the normal maturation of the fetal lung AOE system (8). These *in vivo* findings are consistent with *in vitro* studies by other investigators that demonstrated a trend toward decreases in CAT and GP activities in fetal lung cell cultures after the addition of T_3 to the serum-free medium. The *in vitro* results suggest that T_3 is acting directly on the lung and not via systemic actions or via other serum factors (30). The presence of high-affinity binding sites for thyroid hormone in nuclei of fetal lung cells also suggests that the fetal lung is a direct target tissue for thyroid hormone interaction (31).

The mechanism of the depression by T_3 of the normal late gestational rise in AOE activity is not yet known, *i.e.* whether the findings represent decreased enzyme synthesis, increased enzyme degradation, or inactivation of AOE without direct effects on synthesis or degradation per se (32). Based on the work of Floros *et al.* (33) and Nichols *et al.* (34, 35), who reported decreases in surfactant-associated proteins A, B, and C mRNA in rat lung after T_3 treatment (which still enhanced the synthesis of surfactant phospholipid), we would speculate that TRH treatment (via T_3) may similarly be decreasing gene transcription for the AOE and, ultimately, AOE synthesis (36).

Because neither T_3 nor T_4 crosses the human placenta in significant amounts, two approaches seem feasible to provide the human fetus with the potential benefits of thyroid hormone to stimulate surfactant system maturation in threatened premature delivery. One approach is by intraamniotic instillation of T_3 and T_4. Experimentally, direct *in utero* administration of thyroxine to fetal rabbits results in enhanced lung maturation as judged by lung lavage surfactant content and morphometric studies (37–39). The second approach, stimulating endogenous fetal production of T_3 and T_4 by maternal treatment with TRH (which does cross the placenta), is the approach that is currently being tested clinically. This recent clinical interest in testing the efficacy of prenatal TRH treatment in turn stimulated us to investigate the effects of TRH on both fetal lung biochemical development and newborn lung adaptation to hyperoxia.

In the present study, we investigated in part 1 whether prenatal TRH treatment would act like T_3 on the developing surfactant and AOE systems; we found substantial increases in lung DSPC in late gestation TRH-treated fetuses, as well as significant decreases in CAT, SOD, and GP activities, similar to those found previously with T_3 treatment (8). In addition, to determine whether DEX (which accelerates AOE development) would be able to reverse the effect of TRH (depression of AOE development), we used combined drug therapy and found that although the addition of DEX to TRH resulted in a greater positive effect on surfactant system maturation (increased DSPC), combined DEX plus TRH treatment produced an enhanced negative effect on AOE system development, with further depression of CAT and GP activities in the late gestation fetal lung. This apparent inability of DEX to counteract TRH's depression of AOE system development might be explained if these two hormones act on different cell types or if both TRH and DEX act on the type II pneumocyte (the alveolar lining cell most resistant to hyperoxic exposure) (29, 40–42) but at different sites. It appears from the hormonal measurements in the present study that TRH is working through the secretion of T_3 and T_4 rather than through the release of prolactin, which is consistent with studies previously reported by Klindt *et al.* (43) in fetal lambs as well as the human studies most recently reported by Ballard *et al.* (44).

Hyperoxia studies have consistently revealed that the ability to rapidly increase lung AOE activity during an O_2 challenge correlates with the resistance of the animal to the toxic effects of O_2 on the lung (41, 45, 46). Newborn rats demonstrate this adaptive lung biochemical response and are markedly tolerant to hyperoxic exposure, whereas adult rats are deficient in their

lung AOE responsiveness (showing no AOE increases during >95% O_2 exposure) and are much more susceptible to the toxic consequences of high O_2 exposure. Thus, the ability to respond rapidly to hyperoxic challenge with an increase over the basal levels of enzyme activity appears to be the most important factor in protection of the lung from O_2 free radical injury (47, 48). In line with this concept, in part 2 of our studies, we were able to demonstrate that despite lower AOE levels at the time of birth prenatally treated newborns (TRH plus DEX or TRH alone) were able to mount a normal protective AOE response to hyperoxia, with AOE activity increases of similar magnitude as in the control newborns. All of the neonatal animals were alive after 5 d in >95% O_2. Thus, even though both hormonal treatments produced (worrisome) depressions of baseline protective AOE levels in the newborns, neither TRH alone nor TRH plus DEX pretreatment impaired the newborn lung's capacity to manifest an appropriate biochemical protective response to postnatal high O_2 challenge.

The incidence of respiratory distress syndrome in prematurely born infants has been reduced with the use of prenatal glucocorticoid therapy (49, 50). However, the clinical benefits of glucocorticoids are limited by the number of pregnancies in which steroids have been shown to be ineffective (51, 52). Because of these limiting factors on the clinical efficacy of glucocorticoid treatment, trials of TRH and TRH plus glucocorticoid prenatal therapy have recently been instituted (53, 54). The findings from part 1 of our investigations, which showed significant depression of normal AOE system development with TRH alone or with combined TRH plus DEX treatment, might have raised serious concerns about the clinical use of these agents in prematurely delivered infants who went on to require hyperoxic therapy. However, the results from part 2 of our study have effectively eased these concerns, inasmuch as no detrimental effect on the ability of the newborn animal lung to respond appropriately to hyperoxia was observed after antenatal TRH or TRH plus DEX treatment. The findings in our animal study are somewhat in agreement with the human collaborative studies of Ballard *et al.* (54); although we report substantial increases in lung surfactant content after prenatal hormone therapy, they saw no reduction in the incidence of respiratory distress syndrome. Although there are no studies that definitively demonstrate that AOE regulation is identical in humans and animals, our finding of an enhanced ability to mount a protective AOE response to hyperoxic exposure may correspond to the decreased incidence of chronic lung disease in the studies of Ballard *et al.* (54).

Identification of new agents or drugs that can regulate the normal development of the AOE system or augment these protective enzymes may provide a means to allow for the safer use of high oxygen therapy in neonatal intensive care units.

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