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Propylthiouracil Treatment Decreases the Susceptibility to Oxygen Radical–Induced Lung Damage in Newborn Rats Exposed to Prolonged Hyperoxia

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ABSTRACT. In newborn rats, antenatal thyroid stimulation with thyroid-releasing hormone is associated with developmental decreases in pulmonary antioxidant enzyme activities and decreased survival rates during prolonged hyperoxic exposure, with pathologic evidence of increased O_2-induced lung damage. Propylthiouracil (PTU), in addition to its antithyroid effects, reportedly has antioxidant properties. To explore possible pulmonary protective effects from both the antithyroid and antioxidant properties of PTU, we administered PTU (0.015%) in drinking water to timed-pregnant rats for the final 10 d of gestation and during lactation; control rats received untreated water. The survival rate of the PTU-treated pups when placed in more than 95% O_2 at birth was consistently higher at all time periods in hyperoxia from 6 d [PTU, 81 of 81 (100%); control pups, 70 of 84 (83%); $p < 0.01$] to 14 d [PTU, 41 of 53 (77%); control pups = 14 of 56 (25%); $p < 0.01$]. Further evidence of increased tolerance to more than 95% O_2 in PTU pups included a significant decrease in the incidence of microscopic intraalveolar edema, decreased lipid peroxidation (malondialdehyde), and a significant increase in lung tissue surfactant-related phospholipids compared with O_2-exposed control pups. No differences were present in lung structural maturation, antioxidant enzyme activity response to hyperoxia, or lung tissue O_2 radical formation in more than 95% O_2. We conclude that PTU treatment has important postnatal effects that protect newborn rats against oxidant-induced lung injury and lethality during hyperoxia, which may be related to PTU inhibition of thyroid hormone production, effect on O_2 metabolism, or its direct antioxidant properties. (*Pediatr Res* 35: 530–535, 1994)

Abbreviations

TRH, thyrotropin-releasing hormone
T_3, 3,3′,5-triiodo-L-thyronine
T_4, thyroxine
AOE, antioxidant enzyme
DSPC, disaturated phosphatidylcholine
TPL, total phospholipid
Lm, mean linear intercept (mean air space diameter)
ISA, internal surface area
PTU, propylthiouracil
MDA, malondialdehyde

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Increased production of highly reactive O_2 free radicals has been postulated as a major factor in the development of lung injury during hyperoxic exposure (1). Cell survival under hyperoxic conditions requires that the cell has the capacity to respond to this oxidative stress with an increase in the activity of those defense systems that can detoxify reactive species of O_2 and thereby prevent O_2 toxicity.

Our laboratory has previously demonstrated that fetal thyroid hormone stimulation with the prenatal administration of T_3 or TRH to pregnant rats in late gestation produced lower baseline lung AOE activity levels in their offspring (2, 3). TRH-treated pups demonstrated decreased newborn survival rates during prolonged hyperoxic exposure with evidence of increased lipid peroxidation and intraalveolar edema, all reflective of greater susceptibility to O_2 radical–induced lung damage (4).

PTU is a thioamide derivative that inhibits thyroid hormone synthesis (5). In addition, PTU has recently been reported to be an O_2 radical scavenger and an efficient inhibitor of lipid peroxidation (6). It is currently being used successfully to treat alcoholic liver disease (7) in which chronic ethanol administration has been shown to induce a hepatic hypermetabolic state with increases in hepatic O_2 consumption and O_2 radical production (8).

To explore possible pulmonary protective effects from both the antithyroid and antioxidant properties of PTU, we undertook a series of experimental studies examining hyperoxic survival plus a number of parameters of pulmonary O_2 toxicity in the rapidly growing lungs of newborn animals. On the basis of the previous T_3 and TRH studies, we hypothesized that newborn rats treated with PTU would demonstrate superior tolerance to prolonged high O_2 exposure and an associated decreased susceptibility to O_2 radical–induced lung injury.

MATERIALS AND METHODS

Animals and treatment. Sprague-Dawley albino rats originally obtained from Charles River Laboratories (Wilmington, MA) and maintained in the animal care facilities of the University of Miami School of Medicine under veterinary supervision were used. Breeding was accomplished 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 water and standard rat pellet diet (Rodent Laboratory Chow, Ralston Purina Co., St. Louis, MO) *ad libitum* and kept on a 12-h light/dark cycle.

Ten days before expected delivery of full-term (d 22 of gestation) offspring, pregnant dams were randomly assigned to either a control group or a PTU treatment group. PTU (Sigma Chemical Co., St Louis, MO), 0.015%, was administered in the drink-

ing water, which was made up fresh daily. The control group received drinking water not treated with PTU. With little variability, both PTU and control dams consumed approximately 60 mL water per day. This amount calculates to a dose of ~30 mg/kg/d (approximately twice the directly administered clinical dosage). This regimen has been used previously by others and has been shown to be effective in inhibiting thyroid hormone synthesis without influencing litter size or viability (9).

Newborn rats were obtained by normal parturition within 6–12 h of the beginning of delivery of the first pup. The newborn pups from several equivalently treated litters (PTU or control) were first pooled and then randomly redistributed to the equivalently treated newly delivered dams. Dams plus 10–12 pups/litter were randomly assigned to either a hyperoxic exposure (>95% O_2) or room air exposure group.

Exposures to hyperoxia (96–98%) were conducted in 3.5-cubic-foot clear plastic exposure chambers adapted from regular infant isolettes (model 86; Air Shields, Hatboro, PA) as previously reported (4). The chambers were opened daily (10–15 min) to provide fresh food, water, and cages; to weigh the rat litters; and to interchange mothers between litters exposed to O_2 and to room air to avoid O_2 toxicity in the nursing dams. The offspring were either maintained in hyperoxia for 14 d for survival studies or killed with an overdose of pentobarbital after 5 or 10 d of either hyperoxia (>95% O_2) or room air exposure for the lung analyses described below. The total experimental protocol was preapproved by the University of Miami Animal Welfare Committee.

Lung analyses. Lung lipid peroxidation was assayed by MDA determination according to the method of Buege and Aust (10), after 5 d of more than 95% O_2 exposure. Pups were given an overdose of intraperitoneal pentobarbital, and when no response to a toe pinch could be elicited, a midline abdominal incision was made, the aorta was severed to exsanguinate the animal, and the chest cavity was opened in the midline. The right middle lobe was tied off, and the remainder of the lung was rapidly perfused with ice-cold 50 mM Tris-HCl buffer, pH 7.6 (Sigma) via the pulmonary artery after removing the left atrial appendage to facilitate draining. The right middle lobe was then removed, blotted dry, and placed in a weighed dish for wet/dry measurements. The remainder of the dissected lungs was weighed and homogenized (Brinkmann Polytron Homogenizer, Brinkmann, Westbury, NY) in the Tris-HCl buffer (10:1; vol/wt) with 0.01% of butylated hydroxytoluene. Duplicate aliquots were taken from the homogenate. One sample (unincubated) was placed on ice. We added 50 μL of 2 M ascorbic acid and 50 μL of 0.08 M ferrous sulfate heptahydrate to the samples (incubated), which were then incubated at 37°C for 30 min. To both the unincubated and incubated samples, 0.9 mL of water and 0.6 mL of trichloroacetic acid (28%) were added, and the samples were centrifuged (5000 rpm × 15 min) in a refrigerated centrifuge at 2°C. We added 230 μL of 1% thiobarbituric acid solution to 1600 μL of both supernatants, which were then incubated at 100°C for 15 min. A standard assay curve was generated with MDA tetramethyl acetic acid (Eastman Kodak Co., Rochester, NY), and MDA activity was determined spectrophotometrically at 532 nm as the difference between incubated and unincubated samples and the results expressed as nM MDA/dry lung weight. This method for assessing *in vivo* lipid peroxidation is known to have shortcomings in complex biologic systems [including the cross-reactivity of thiobarbituric acid with different commonly occurring cytosolic compounds (11)]. Nonetheless, it provides a generally acceptable and frequently used indirect means for assessing comparative hyperoxia-related lung damage.

Oxygen radical production was indirectly determined by the rate of cyanide-resistant O_2 consumption with the method of Freeman *et al.* (12), with minor variations. Rat lungs were perfused *in situ* with 10 mM potassium phosphate buffer, pH 7.4, and homogenized in 50 mM potassium phosphate buffer (15:1; vol/wt) for 30 s at full speed with an Omni 2000 homogenizer (with a 10-mm probe; Omni International, Inc., Waterbury, CT). After duplicate portions were removed for DNA analysis, the samples were centrifuged at 600 × *g* for 10 min to remove nuclei, cells, and connective tissue debris. The supernatant fluid was then used to measure O_2 consumption. To 1.5 mL of sample, 1.5 mL 50 mM potassium phosphate buffer and 30 μL of 1 mM NADH were added, and the mixture was then equilibrated with air at 30°C. The rate of O_2 consumption was measured with a YSI model 5300 oxygen monitor equipped with standard bath and electrodes (Yellow Springs Instrument Company, Inc., Yellow Springs, OH) and a flatbed recorder. The electrode was then removed, 30 μL of 100 mM NaCN added, the mixture reequilibrated with air, and the rate of cyanide-resistant O_2 uptake measured. The rate of O_2 uptake was expressed as nmol O_2 consumed per min per mg DNA. Addition of cyanide caused a 2- to 2.5-fold reduction in the rate of O_2 consumption.

For lung AOE activity analysis after 5 d of more than 95% O_2 or room air exposure, newborn pups from each group were killed as described above, and their lungs were immediately perfused *in situ* with ice-cold saline and then homogenized in cold saline (20–30:1; vol/wt). Two to three lungs were pooled per sample to provide adequate lung tissue for the assays. The homogenates were frozen at −70°C for subsequent AOE analyses. Lung activities of total superoxide dismutase (13), catalase (14), and glutathione peroxidase (15) were assayed by standard spectrophotometric techniques. Purified enzyme standards (superoxide dismutase and catalase) were obtained from Sigma Chemical; glutathione peroxidase standard was obtained from Boehringer-Mannheim Co. (Indianapolis, IN). Lung protein was determined with purified bovine albumin (Sigma) as standard (16), and lung DNA was determined with purified calf thymus DNA (Sigma) as standard (17).

For phospholipid determinations, aliquots of the saline-perfused lung homogenates were subjected to lipid extraction according to the procedure of Bligh and Dyer (18). The lipid extract, once dried under nitrogen, was frozen at −70°C before phospholipid analysis. An aliquot of lipid extract was designated for measurement of TPL (19). A second aliquot was used for quantitating DSPC, using the method of Mason *et al.* (20). 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 (19). A known quantity of ^{14}C-dipalmitoyl-phosphatidylcholine (New England Nuclear,

Table 1. *Comparative parameters in PTU and control rat offspring after 5 d in >95% O_2 or room air**

Treatment group	Body wt (g)	Lung wt (g)	Lung wt/body wt (%)	Protein (mg/g lung)	DNA (mg/g lung)	Protein/DNA
Air control	12.20 ± 1.85	0.239 ± 0.045	1.81 ± 0.20	68.40 ± 12.37	9.38 ± 2.24	7.48 ± 1.33
Air PTU	10.76 ± 1.12†	0.185 ± 0.026†	1.70 ± 0.15	62.31 ± 11.20	8.58 ± 2.07	7.65 ± 1.78
O_2 control	12.13 ± 1.55	0.218 ± 0.039	1.75 ± 0.28	69.05 ± 12.56	6.92 ± 2.42§	11.45 ± 2.46§
O_2 PTU	10.26 ± 1.14‡	0.151 ± 0.016‡	1.42 ± 0.13‡	61.00 ± 13.83	7.84 ± 2.56	7.65 ± 1.78‡

* Values are means ± 1 SD for four to nine litters per group.

† Statistically significant with $p < 0.05$ for air PTU *vs* air control.

‡ Statistically significant with $p < 0.05$ for O_2 PTU *vs* O_2 control.

§ Statistically significant with $p < 0.05$ for O_2 control *vs* air control.

Table 2. *Serum T_3 and T_4 levels in PTU-treated and control offspring after 10 d in >95% O_2 or room air**

Treatment group	T_3 (nmol/L)	T_4 (nmol/L)
Air control	0.447 ± 0.201	17.23 ± 5.35
Air PTU	<0.138†	<10.1†
O_2 control	0.503 ± 0.168	17.92 ± 6.80
O_2 PTU	<0.138†	<10.1†

* Values are means ± 1 SD for three litters, five to eight samples per group (assay lower limits = 0.138 nmol/L for T_3 and 10.1 for T_4).

† Statistically significant with $p < 0.05$ for all PTU-treated *vs* all control groups.

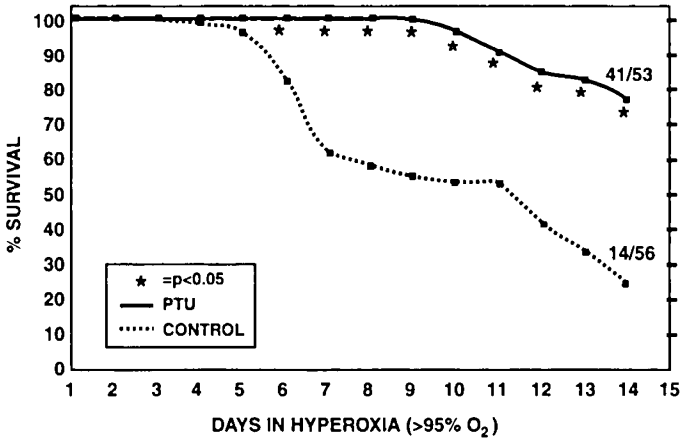

Fig. 1. Survival of PTU-treated and control newborns in >95% O_2 for 14 d (*n/n* = number alive/number put in O_2). The PTU-treated pup survival rate is significantly increased compared with O_2 controls at all time periods from 6 to 14 d in hyperoxia. *, $p < 0.01$, χ^2.

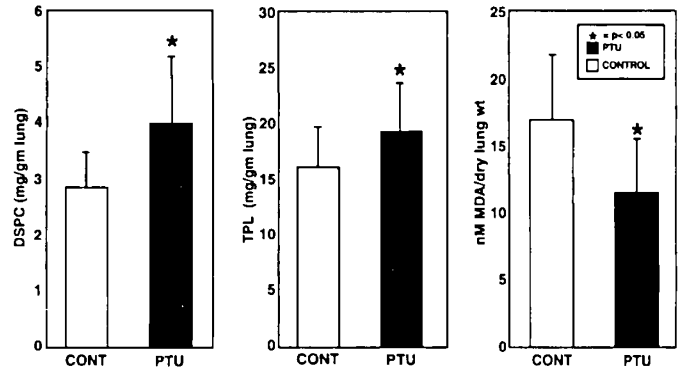

Fig. 2. Lung tissue DSPC, TPL, and MDA content in PTU and control (*CONT*) rat offspring in >95% O_2 for 5 d. Values are mean ± 1 SD for three to five litters per group, eight to 19 samples per group. The air control *vs* air PTU values for DSPC, TPL, and MDA are, respectively, 3.28 ± 0.63 *vs* 3.35 ± 0.64 mg/g lung, 18.58 ± 3.34 *vs* 18.07 ± 3.51 mg/g lung, and 12.28 ± 5.39 *vs* 7.39 ± 1.56 nM MDA/g dry lung weight. *, $p < 0.05$ for PTU compared with control group.

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 weight (and also calculated per mg of protein and as a ratio of mg DSPC to mg TPL).

Microscopic studies. For microscopic studies, the pups from each group that had been killed had their lungs inflated *in situ* through a tracheal catheter at a constant 20 cm H_2O pressure (fixative: 10% buffered formalin). Fixation was continued in formalin at room temperature for 48 h before determination of lung volume by water displacement (21) and sectioning. From all lungs, similarly oriented sections from similar portions of the left lung and the right middle and lower lobes were stained with

Table 3. *Comparative pulmonary antioxidant enzyme activities in PTU and control rat offspring after 5 d in >95% O_2 or room air**

Treatment group	SOD	CAT	GP
Air control	78.8 ± 26.4	309 ± 85	0.293 ± 0.038
Air PTU	92.3 ± 16.0	303 ± 70	0.334 ± 0.045
O_2 control	110.0 ± 26.0†	403 ± 132†	0.501 ± 0.083†
O_2 PTU	108.1 ± 22.7	389 ± 95†	0.538 ± 0.086†

* Values are means ± 1 SD for four litters, 14 to 15 samples per group. Antioxidant enzymes: superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GP) are expressed as activity units/mg DNA.

† Statistically significant with $p < 0.05$ for respective O_2-exposed *vs* air-exposed groups.

hematoxylin and eosin and initially examined to eliminate any section with evidence of inadequate preparation (atelectasis). No correction was made for tissue shrinkage. Light level morphometric assessment was performed on coded slides for comparison of lung structural development (alveolarization) in air and O_2-exposed neonatal animals from all experimental groups. We determined Lm, percentage air space/percentage tissue space, ISA, and specific ISA (ISA/100 g body weight) with a standard integrating eyepiece with seven horizontal lines and 42 intercept bars (Zeiss Optical, Oberkochen, Germany). The coded slides were examined at ×400 magnification by two investigators, with 30 random fields counted per slide. We counted the number of times the intercept bars fell on lung tissue septa per field and the number of transections of airspace walls with the seven horizontal lines per field. To calculate Lm (representing the average distance between air space walls, or mean air space diameter), we used the following formula: Lm = (length of line × number of lines counted × number of fields)/total number of tissue transections, where the length of each line = 0.20 mm, number of lines = 7, and number of fields = 30 (22).

ISA, representing the internal surface area of the lung available for respiratory exchange, was calculated with the formula: ISA = (4 × lung volume)/Lm, where lung volume is the determined postfixation lung volume (23). Percentage air space was calculated as: % air space = Pa/(Pa + Pt) × 100, where Pa is the total number of intercept bars hitting air and Pt is the total number of intercept bars hitting tissue.

Pulmonary edema was microscopically assessed in coded lung sections by evidence of interstitial or peribronchial-perivascular swelling and pink-staining (proteinaceous) material within the air spaces (intraalveolar edema). Pulmonary edema was also assessed by comparative wet/dry lung weights using nonperfused lung lobes weighed before and after drying in an 80°C oven for 48–72 h to reach constant weight.

Serum hormone assay. From randomly selected PTU-treated and control 10-d-old offspring, hormone assays for serum T_3 and T_4 were performed with specific RIA kits (Incstar Corporation, Stillwater, MN). The assay sensitivities for T_3 and T_4 were 0.138 nmol/L and ≤10.1 nmol/L, respectively.

Statistical analysis. Survival rates of the treated *versus* untreated rat pups and assessment of intraalveolar edema were compared by χ^2 testing (24). For comparing biochemical values for the two hyperoxic groups with those of the two air control groups, one-way analysis of variance was performed, followed by Duncan's multiple range test (24). For all statistical tests, a $p < 0.05$ value was considered to represent a significant difference between the compared values.

RESULTS

Physical characteristics. The influence of prenatal PTU on body weight was determined at birth, and postnatal PTU influence was determined after 5 d in air or hyperoxia. Body weights were slightly (−5%) but significantly decreased at birth in the PTU-treated offspring (6.31 ± 0.41) compared with the control

Table 4. *Comparative morphometry of newborn rat pup lungs after 10 d in >95% O_2 or room air**

Treatment group	Lung volume/100 g	Airspace (%)	Lm (μm)	ISA (cm^2)/100 g
Air control	4.08 ± 0.38	71.4 ± 3.5	46.7 ± 2.9	3506 ± 429
Air PTU	3.94 ± 0.45	74.8 ± 1.8	51.0 ± 2.6	3103 ± 357
O_2 control	4.36 ± 0.34	79.2 ± 2.1†	72.8 ± 10.7†	2465 ± 279†
O_2 PTU	3.76 ± 0.62	78.1 ± 3.7	73.9 ± 5.9†	2024 ± 194†

* Values are mean ± 1 SD for four to six rat lungs per group.

† Statistically significant with $p < 0.05$ for respective O_2-exposed group *vs* air-exposed group.

offspring (6.63 ± 0.62) ($p < 0.05$) and remained significantly decreased after 5 d in air (−12%) or in hyperoxia (−15%) (Table 1). Lung wet weights were also significantly decreased after 5 d in air or hyperoxia in the PTU-treated offspring, as were the lung weight/body weight ratios in hyperoxia (Table 1).

PTU treatment followed by 5 d in air or hyperoxia did not result in significant changes in the normal rate of increase of lung protein or DNA content associated with the maturation process. The greater protein/DNA ratio in the O_2 control group (Table 2) largely reflects a significant degree of DNA inhibition in the O_2 control group lungs (−26% *versus* respective air control), which was not seen in the O_2 PTU group lungs O_2 (−8% *versus* respective air PTU).

Survival data. The offspring treated with PTU demonstrated a significantly superior survival rate compared with the control offspring from the 6th d onward in hyperoxia, with the comparative 14-d-survival rate being 3 times greater (77%) for the O_2 PTU group *versus* the O_2 control group (25%) (Fig. 1).

Serum hormone assays. The T_3 and T_4 serum levels in PTU-treated *versus* control offspring after 10 d of either room air or hyperoxic exposure are listed in Table 2. PTU consistently inhibited T_3 and T_4 levels.

Lung analyses. The lung tissue surfactant-related phospholipids (DSPC and TPL) and MDA content in PTU and control rat offspring after 5 d of hyperoxia are illustrated in Figure 2 (values for 5-d air-breathing controls with and without PTU are provided in the legend). Offspring of PTU-treated dams had significant increases in lung tissue DSPC and TPL compared with control offspring in hyperoxia and a significant decrease in MDA per dry lung weight, reflecting an inhibition of *in vivo* lipid peroxidation.

Comparative pulmonary AOE activity responses after 5 d of hyperoxia were essentially similar between the O_2-PTU *versus* O_2-control groups (Table 3). Likewise, measurements of total O_2 consumption or cyanide anion–resistant O_2 consumption as a reflection of O_2 radical production were not different between the PTU (4.0 ± 0.4 nmol O_2 consumed/min/mg DNA) and control (4.3 ± 0.3 nmol O_2 consumed/min/mg DNA) O_2-exposed groups.

Microscopic studies. The comparative lung morphometric findings for PTU-treated and control offspring after 10 d of hyperoxia are shown in Table 4. The improved survival in hyperoxia of PTU offspring occurred despite no apparent effect on the magnitude of O_2-induced inhibition of normal lung structural maturation associated with hyperoxic exposure. The Lm values were increased similarly (~50%) in both O_2 groups, and comparable decreases (~30%) were observed in normal ISA development in hyperoxia. Quantitative morphometric studies were also carried out on offspring after 5 d of hyperoxia with no significant differences between PTU and control offspring (data not shown).

Qualitative examination of the same coded slides used for quantitative morphometry revealed that all the O_2-exposed pups had evidence of perivascular or peribronchiolar edema present after 5 and 10 d of hyperoxic exposure. This microscopic finding was further substantiated by the increased wet/dry lung weights of the O_2-exposed offspring *versus* the offspring maintained in room air. After 5 d in hyperoxia, no differences were observed in wet/dry lung weights between the PTU (6.10 ± 0.59) and control (6.16 ± 0.79) offspring (average air control = 5.37 ± 0.23). However, when pathologic evidence of more advanced O_2 toxicity was examined, intraalveolar edema was present in 47% (14 of 30) of the control group lung sections after 5 d of hyperoxia *versus* only 7% (2 of 30) of the PTU group lung sections ($p < 0.01$). By 10 d in hyperoxia, 14% (3 of 21) of the PTU group lung sections had evidence of intraalveolar edema fluid compared with 59% (10 of 17) of the control group lung sections ($p < 0.01$). The similar increases in wet/dry weights in the O_2 groups suggest endothelial cell injury was present in both groups. However, the intraalveolar edema results would seem to suggest a greater degree of injury to the alveolar-epithelial cell barrier of the O_2-control group.

DISCUSSION

Hyperoxia upsets the normal cellular oxidant-antioxidant defense equilibrium by producing marked increases in O_2 free radical production (25). The newborn animal's superior ability to resist O_2-induced lung damage (and lethality) compared with adult animals appears to be at least partly related to the newborn's ability to increase its basal AOE activity levels in response to more than 95% O_2 hyperoxia, a biochemical response adult animals do not demonstrate (26–29). We had previously shown that after prenatal TRH treatment, despite lower baseline lung AOE activity levels, the TRH newborn rats were able to increase their baseline AOE activities markedly in response to hyperoxia (3). Unexpectedly, however, TRH offspring were found to have comparatively poorer survival in hyperoxia with increased indices of O_2 radical–induced lung damage compared with control newborns (4). Because the maturation of the rat hypothalamic-pituitary axis normally occurs relatively late in the neonatal period (30–32), the detrimental effects of TRH treatment on O_2 tolerance during the first 2 wk of life may have been related to a relative hyperthyroid state in the TRH-offspring compared with a relative hypothyroid state in control neonatal rats (with immature pituitary-hypothalamic function) (33). Significantly elevated T_3 and T_4 serum levels were in fact found in the 10-d-old prenatally TRH-treated offspring *versus* control 10-d-old rat pups (4). We speculate that this relative hyperthyroidism in TRH offspring may have enhanced basal metabolic rate, increased O_2 consumption, and increased O_2 radical production (34), which could have exceeded the detoxifying capacity of the antioxidant defense mechanisms and produced increased pulmonary O_2 toxicity and reduced survival in hyperoxia. We undertook the present study to test the effect of inhibited endogenous thyroid hormone production (by PTU treatment) on the O_2 tolerance of the newborn rat.

PTU is a thioamide derivative that is known to cross the placenta, inhibit thyroid hormone synthesis by blocking the incorporation of iodine, and also inhibit the peripheral deiodination of T_4 to T_3 (5). The serum hormone data (Table 2) reflect the effectiveness of maternal PTU treatment in the fetus and neonate when it is administered in the maternal rat's water source. Multiple investigations (6, 35) have recently reported that PTU, in addition to its antithyroid properties, has the ability to protect membrane lipids from peroxidative directly changes, the potential to scavenge free radicals directly (36), diminish H_2O_2 production (37), reduce O_2 radical production indirectly by

decreasing the production of inflammatory mediators (38), and decrease O_2 consumption and O_2 radical production by its antithyroid actions (39). In fact, PTU is currently being used effectively to treat severe alcoholic liver disease (7), a hepatic hypermetabolic state with increases in hepatic uptake of T_4, increased hepatic O_2 consumption, and excess superoxide radical production by hepatic microsomal fractions (8). Recently, patients treated with PTU have been found to have decreased serum levels of lipid peroxides, suggesting that PTU used clinically may scavenge O_2 free radicals or may detoxify peroxyl radicals to prevent chain reaction lipid peroxidation (40).

The overall results of our newborn rat PTU treatment protocol represent the logical reversal of the results found previously in our prenatal TRH treatment study (4). In addition to the consistent and dramatically superior survival rate of the PTU-treated offspring in hyperoxia (compared with the poorer survival of TRH offspring), we found concomitant evidence of increased protection against O_2 radical–induced lung damage in the MDA and DSPC/phospholipid data (Fig. 2), in the comparative intraalveolar edema results, and in the reduced inhibition of lung DNA (synthesis) during O_2 exposure. The decrease in MDA may reflect not only a decrease in oxidative stress and lipid peroxidation but also possibly a decrease in the quantity of polyunsaturated lipids available for peroxidation. Hoch *et al.* (41), for example, reported that in rats made hypothyroid by thyroidectomy, their livers incorporated less linoleate (18:2) into unsaturated fatty acid (especially 20:4, arachidonic acid) and instead incorporated the label mainly into saturated fatty acids, suggesting that hypothyroidism may decrease the quantity of polyunsaturated lipids available for peroxidation. This finding is in agreement with the study of Fernandez *et al.* (8) in adult rats, in which T_3 treatment produced significant elevations of hepatic MDA levels *versus* untreated euthyroid rats. It is also conceivable to postulate that because PTU is soluble in a hydrophobic environment (6), the decrease in MDA may represent PTU's equilibration into surfactant-related phospholipids or membranes of the alveolar epithelium to act there as a chain-breaking antioxidant.

Our findings are in agreement with the study of Yam and Roberts (42) in adult rats, in which they reported a decrease in hyperoxic lethality and pulmonary edema after pretreatment with PTU. However, they also reported a significant increase in baseline pulmonary AOE activities with PTU treatment. Chen *et al.* (43), in rat pups prenatally treated with thyroid hormone, demonstrated a significant inhibition of the normal elevations in baseline AOE activity in the late gestational fetal lung caused by a direct negative regulation of AOE gene expression at the transcriptional level. Our comparative AOE levels at 5 d of life were not different between the PTU and control pups (Table 3), which is consistent with our previous work illustrating no difference in AOE activities at birth after thyroid inhibition (by PTU) when compared with control newborn rats (44). We were also unable to find the decrease in lung tissue cyanide anion–resistant O_2 consumption and O_2 radical formation that we anticipated in our PTU-treated animals, suggesting that either our (indirect) method was not sensitive enough to detect small but meaningful changes in O_2 radical formation with PTU treatment or that PTU by its antioxidant action fosters the enhanced removal of free radicals in the neonatal lung without affecting O_2 free radical production per se.

Whether PTU's marked protective effect against O_2 toxicity in the newborn rat stems from its antithyroid properties and its effect on O_2 metabolism or its direct antioxidant properties requires further exploration. It is conceivable that PTU treatment could protect the lungs of premature infants from the acute and chronic toxic damage associated with required O_2 administration.

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