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Synergistic Effect of Triiodothyronine and Dexamethasone on Male and Female Fetal Rat Lung Surfactant Synthesis[1]

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Abstract. It has previously been reported that morphologic and functional indices of fetal lung maturation are delayed in male rabbit fetuses, and these differences cannot be overcome by glucocorticoid treatment. Since thyroid hormone also stimulates surfactant production we have studied the effects of triiodothyronine (T_3) and dexamethasone on saturated phosphatidylcholine (SPC) synthesis by day 20 male and female rat lung. Control male lung slices incorporated significantly less ^{3}H-choline into SPC than females; surgery on day 17 markedly stimulated ^{3}H-choline incorporation into SPC by both sexes, but the sex difference persisted. The optimal dose of T_3 inhibited SPC synthesis by both sexes while dexamethasone was stimulatory. When T_3 and dexamethasone were given together they had a greater effect than that of dexamethasone or T_3 alone. Based on these results we conclude that the male deficit in pulmonary surfactant production may be overcome by the synergistic effects of T_3 and dexamethasone.

Introduction

The risk of death due to respiratory distress syndrome (RDS) in the newborn period is significantly higher in the male than in the female [1]. This is probably due to the production of less pulmonary surfactant by the male since there is significantly less surfactant-associated phosphatidylcholine in amniotic fluid from males than from females during the last trimester of human gestation [2] and there is less surfactant-associated phosphatidylcholine in male fetal rabbit lung lavage and amniotic fluid than in female during late gestation [3]. With the advent of methods for the prediction of

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RDS risk and the use of glucocorticoids to augment surfactant production antenatally the risk of RDS has been significantly reduced. However, such treatment remains relatively ineffective in the male [4].

Fetal lung maturation is regulated by an ever-expanding list of hormones [5]. The most widely recognized stimulators of pulmonary surfactant production are glucocorticoids and thyroid hormone. *Smith and Sabry* [6] have recently observed that triiodothyronine (T_3) augments the effect of glucocorticoids on surfactant synthesis, but has no effect of its own in tissue culture. We tested the hypothesis that the synergistic effect of T_3 would override the sex difference in surfactant synthesis.

Methods and Materials

Time-mated Sprague-Dawley rats were purchased from Charles River Breeders, Wilmington, Mass. On day 17 laparotomy was performed on rats under ether anesthesia. The uterus was exteriorized and either saline, dexamethasone, T_3 or dexamethasone and T_3 was intra-amniotically administered to each of the pups in one uterine horn, using a 50-μl Hamilton syringe and a 26-gauge needle, leaving the contralateral horn uninjected or saline-treated. The uterus was replaced and the abdomen was sutured. The mothers were killed on day 20 with chloroform and the fetuses were removed and decapitated to prevent expansion of the lungs. All fetuses were weighed, the sex was determined by inspection of the gonads [7] and the lungs were removed en bloc, weighed, and left on ice in Krebs-Ringer bicarbonate buffer, pH 7.4. Lung slices were assayed for the rate of phosphatidylcholine synthesis by a modification of the method of *Farrell and Epstein* [8] as follows. The lungs were cut into 0.5-mm slices using a MacIlwain tissue chopper. Approximately 100 mg wet weight lung tissue was put into 20-ml vials containing 4 ml of iced Krebs-Ringer bicarbonate buffer, pH 7.4. The vials were incubated at 37 °C under CO_2:O_2 (5:95) for 10 min in a Dubnoff metabolic shaker. At this point, 2 μCi of choline chloride [methyl-^{3}H] in 100 μl ethanol were added to each vial and the incubation was allowed to proceed for an additional 60 min. At the end of the incubation period the vials were placed on ice, an excess of iced saline was added to each vial and the vials were left for 5 min. The incubation mixture was then filtered through nylon mesh, washed 3–4 times with 5 ml of iced saline and the tissue was placed in vials and frozen in liquid nitrogen. A 10% (weight:volume) homogenate of the tissue was made with saline and sonicated from 30 s to 1 min. An aliquot of the sonicate was extracted by the method of *Folch* and the extract was chromatographed on silica gel H in the solvent system $CHCl_3$:MeOH:H_2O (65:25:4). Another aliquot was treated with osmium tetroxide by the method of *Mason* et al. as modified by *Torday* et al. [9]. The developed chromatograms were stained with bromthymol blue and the phosphatidylcholine and saturated phosphatidylcholine (SPC) areas were cut out and put into liquid scintillation vials containing 10 ml of Atomlite (NEN, Boston, Mass.) to determine their radioactive content.

Aliquots of the sonicate were taken to determine protein content by the Lowry method [10]. Data were analyzed statistically by a three-way analysis of variance and Student's t test.

Results

SPC represented 25–40% of the phosphatidylcholine synthesized from ^{3}H-choline under our experimental conditions. In fetuses from unmanipulated rat mothers the rate of SPC synthesis was 25% higher in females than in males ($p < 0.01$) (table III). In fetuses from rat mothers which had been operated on day 17 of gestation and injected with 0.1 ml of 0.9% saline there was a 70% increase in SPC synthesis in both sexes, and the sex difference persisted ($p < 0.06$) (table III).

Dexamethasone was tested at three doses. At 0.5 μg/fetus there was no effect on the rate of SPC synthesis (table III) or on lung and body weights (table I, II). 1 μg of dexamethasone/fetus caused a 12% increase in ^{3}H-choline incorporation into SPC in female fetuses and a 27% increase in males, resulting in an elimination of the sex difference; at this dose of dexamethasone there was no effect on either lung or body weight ($p > 0.07$) (table I, II). There was no effect of dexamethasone on fetal wastage at either

Table I. 20-day fetal body weight

	Uninjected control	Saline	Dexamethasone	T_3	Dex + T_3
Male	2.32 ± 0.03 15[1]	2.33 ± 0.03 56	2.36 ± 0.08 (0.5)[2] 19	2.30 ± 0.03 (0.1) 10	2.39 ± 0.09 (1, 0.1) 8
			2.40 ± 0.05 (1.0) 37	2.34 ± 0.03 (1.0) 18	2.37 ± 0.07 (1, 1) 24
			2.23 ± 0.09 (2.0)* 16		
Female	2.28 ± 0.01 27	2.30 ± 0.05 46	2.40 ± 0.10 (0.5) 16	2.32 ± 0.03 (0.1) 10	2.30 ± 0.06 (1, 0.1) 12
			2.33 ± 0.03 (1.0) 47	2.32 ± 0.04 (1.0) 18	2.25 ± 0.04 (1, 1) 21
			2.12 ± 0.05 (2.0)* 22		

Each value represents the mean ± SEM fetal body weight in grams of fetuses obtained from at least 2 litters. Statistical differences were determined by a 3-way analysis of variance. *Saline versus treated, $p < 0.01$.

[1] Number of fetuses.

[2] Drug dosage in micrograms.

of these doses. When a dose of 2μg/fetus was tried there was increased fetal morbidity and mortality (results not shown) and inhibition of ^{3}H-choline incorporation into SPC (table III).

T_3 was also tested at three doses. At 0.1 μg/fetus there was no effect on either the rate of ^{3}H-choline incorporation into ^{3}H-SPC (table III), or on body and lung weights (table I, II). At 1.0 μg/fetus there was significant inhibition of ^{3}H-choline incorporation into ^{3}H-SPC in both male (43%) and female (53%) pups ($p < 0.0001$). There was also significant reduction in both body and lung weight ($p < 0.01$). Treatment with 2 μg of T_3/fetus resulted in greatly increased fetal mortality (results not shown).

When fetuses were treated with 1 μg each of dexamethasone and T_3 together there was a significant increase in the rate of ^{3}H-choline incorporation into ^{3}H-SPC in both male (32%) and female (28%) fetuses (table III) when compared to dexamethasone or T_3 treatment alone. There were no effects in either lung or body weights in these animals (table I, II). Dexa-

Table II. 20-day fetal lung weight

	Uninjected control	Saline	Dexamethasone	T_3	Dex + T_3
Male	103 ± 4 27[1]	101 ± 2 42	104 ± 4 (0.5)[2] 24	101 ± 1 (0.1) 26	100 ± 5 (1, 0.1) 22
			110 ± 6 (1.0) 26	84 ± 1 (1.0)* 35	94 ± 4 (1, 1) 29
			76 ± 2 (2.0)* 13		
Female	101 ± 2 18	96 ± 2 30	103 ± 5 (0.5) 28	98 ± 3 (0.1) 32	99 ± 5 (1, 0.1) 34
			113 ± 10 (1.0) 22	82 ± 1 (1.0)* 34	88 ± 5 (1, 1) 16
			68 ± 3 (2.0)* 15		

Each value represents the mean ± SEM fetal lung weight in milligrams of fetuses obtained from at least 2 litters. Statistical differences were determined by a 3-way analysis of variance. *Saline versus treated, $p < 0.01$.

[1] Number of fetuses.

[2] Drug dosage in micrograms.

methasone (1 μg/fetus) was also injected in combination with 0.1 μg of T_3/fetus, but there was no effect compared to dexamethasone given alone at this dose (table III).

Discussion

Glucocorticoids have been used clinically to prevent RDS for over a decade [11]. Though it has long been recognized that they are only about 50% effective in preventing RDS [12] it was not known why this treatment failed. The recent multicenter trial of glucocorticoid therapy has indicated that the drug may be ineffective in males [4]. Studies from our laboratory have established that there is a sex difference in prenatal surfactant production in man [2] and rabbit [3]. This lag in male fetal surfactant production is likely due to circulating fetal androgens since (a) dihydrotestosterone inhib-

Table III. 20-day fetal lung slice ^{3}H-choline incorporation into ^{3}H-SPC

	Control	Saline	Dexamethasone	T_3	Dex + T_3
Male	1,350 ± 75 17[1]	2,400 ± 100 23	2,025 ± 50 (0.5)[2] 7	2,150 ± 125 (0.1) 10	2,500 ± 100 (1, 0.1) 3
			3,050 ± 125[b] (1.0) 25	1,350 ± 75[b] (1.0) 12	4,050 ± 75[b,c] (1, 1) 12
			2,375 ± 50 (2.0) 8		
Female	1,700 ± 100* 17	2,925 ± 100[a]* 21	2,825 ± 425 (0.5) 8	2,700 ± 150 (0.1) 10	2,375 ± 125 (1, 0.1) 3
			3,300 ± 275[b] (1.0) 25	1,350 ± 75[b] (1.0) 12	4,225 ± 75[b,c] (1, 1.0) 11
			2,175 ± 200 (2.0) 9		

Each value represents the mean ± SEM dpm/mg protein of fetuses obtained from at least 2 litters. Statistical differences were determined by a 3-way analysis of variance. * Male versus female, $p < 0.01$. a = Saline versus uninjected control, $p < 0.06$; b = treated versus saline, $p < 0.01$; c = Dex or T_3 versus Dex + T_3, $p < 0.01$.

[1] Number of fetuses.

[2] Drug dosage in micrograms.

its this process, (b) the antiandrogen flutamide abolishes the sex difference, and (c) endogenous androgens from male fetuses inhibit female fetal surfactant production [13]. In addition, we have found that this sex difference in surfactant production is also due to genetic factors since it is reversed in the chick embryo in which the male is homogametic, not the female [14]. However, the clinical problem of overcoming this male deficit remains unresolved. *Ballard* et al. [15] had shown that glucocorticoids cross the placenta efficiently in both male and female, are equally effective in suppressing pituitary-adrenal function, and that there is no sex difference in adrenocortical function. Therefore, since it appears that the response to glucocorticoid by the male fetal lung was blunted, we reasoned that perhaps a combination of surfactant stimulants might overcome the male deficit.

In the present study we have demonstrated that there is a sex difference in the rate of choline incorporation into SPC by male fetal rat lung. Treatment with dexamethasone eliminated this sex difference, unlike that seen in the clinical experience. This may be due to species difference or the late stage of development at which the fetuses were treated. T_3 alone had no beneficial effect on choline incorporation into SPC unlike previous studies with T_4 [16] and DIMIT [17], a synthetic analogue of T_3. This may be due to differences in the half-lives of T_3 and T_4 [18]. When T_3 was administered in conjunction with dexamethasone it produced an effect in both sexes which was greater than that of dexamethasone alone. These results are similar to those reported by *Hitchcock* [19] who found synergistic effects of thyroid hormone and glucocorticoid on morphologic maturation of fetal rat lung, though the sexes of the fetuses were ignored. Based upon these observations we conclude that the male deficit in surfactant production can be overcome by administration of T_3 in combination with dexamethasone.

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