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Glucocorticoid-Thyroid Hormone Interactions in Fetal Rat Lung⁽¹³⁾

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Summary

Previous studies have shown that triiodothyronine (T_3) enhances the effect of dexamethasone on phosphatidylcholine (PC) synthesis in organ cultures of fetal rat lung. The aim of this study was to investigate whether similar interactions occurred *in vivo* and to explore possible mechanisms for this phenomenon.

Injection of 7.0 mg/kg T_3 into pregnant rats on d 18 and 19 of gestation resulted in a mean fetal serum T_3 level of 2380 ng/dl on d 20 (control, 84 ng/dl) and in maximal (34%) stimulation of choline incorporation into PC. Injection of 1.0 mg/kg betamethasone using the same protocol as for T_3 resulted in maximal stimulation of 33% and administration of both hormones together produced a 69% increase, an additive effect. The percentage of PC that was disaturated was increased with betamethasone, but decreased with T_3 . Betamethasone treatment resulted in an increase in the whole lung disaturated PC content, but treatment with T_3 did not. Betamethasone administration also increased fetal serum T_3 levels, but T_3 injection did not produce elevated fetal serum corticosterone levels.

Injection of T_3 *in vivo*, or exposure of explants of 18-d fetal lung to 100 nM T_3 for up to 48 h did not result in an increase in cytoplasmic glucocorticoid binding or nuclear translocation of the receptor steroid complex. Exposure of explants to glucocorticoid or T_3 *in vivo* or in culture (dexamethasone, 100 nM and T_3 , 100 nM; for 48 h) resulted in a significant increase in the activity of cholinephosphate cytidyltransferase, an enzyme in the choline incorporation pathway of PC synthesis. Exposure of explants to the combination of hormones resulted in stimulation that was equal to the sum of that produced by the single hormones but was not statistically significantly different from the glucocorticoid effect. The activities of other enzymes of phospholipid synthesis were not increased by exposure to either hormone, *in vivo* or *in vitro*.

The additive effects of T_3 and glucocorticoid with regard to choline incorporation into PC in fetal rat lung suggest that combined hormone therapy may be useful for the prevention of

respiratory distress syndrome in humans. Further animal studies are required, however, before clinical use can be considered.

Abbreviations

CDP, cytidine 5' diphospho
DIMIT, 3,5-dimethyl-3'-isopropyl-L-thyronine
PC, phosphatidylcholine
RDS, respiratory distress syndrome
 T_3 , triiodothyronine

Interactions between glucocorticoids and thyroid hormones occur in a number of tissues (31). A previous study from this laboratory showed that there are additive interactions between dexamethasone and T_3 with regard to phospholipid synthesis in explants of fetal rat lung in organ culture (11). Both dexamethasone and T_3 stimulated the rate of choline incorporation into PC in these cultures and the effect of the combination of these hormones was equal to or greater than the sum of their individual actions. Another of our previous studies indicated that glucocorticoids and thyroid hormones have different effects on fetal lung phospholipid metabolism, suggesting that these hormones act at different biochemical sites (10).

The aim of this study was 2-fold. First, we wanted to determine whether the interactions observed *in vitro* would occur *in vivo*. The second goal was to determine whether the interactions occur at the level of cytoplasmic glucocorticoid receptor, nuclear translocation of the receptor-steroid complex, or at the translational level. To explore this, we examined the effects of T_3 on dexamethasone binding, *in vivo* and *in vitro*, and the effects of glucocorticoids, T_3 , and a combination of these two hormones on the activity of a number of enzymes of phospholipid synthesis.

MATERIALS AND METHODS

In vivo studies. In order to avoid problems associated with direct fetal injection, all hormones were injected into the mother.

Timed pregnant Sprague Dawley rats (Charles River Breeding Laboratories, Cambridge, MA) were injected once in the morning on d 18 and 19 of gestation with either saline, betamethasone, T_3 in 0.9% saline, or a combination of the above hormones. The rats were killed by decapitation on d 20 and the fetuses removed under sterile conditions. One-half of the fetuses was used for enzyme assays and the other half was weighed, decapitated, and bled. The serum was retained for analysis of hormone levels. The lungs were then removed, dissected free of non-pulmonary tissue and large airways, and chopped into 1-mm³ cubes by means of a mechanical tissue chopper. The lung pieces from fetuses from the same litter were pooled and used for measurement of the rate of choline incorporation into PC and disaturated PC, as described previously for lung explants (9), and determination of phospholipid content or glucocorticoid binding.

Organ cultures. All experiments were performed with explants of 18-d fetal rat lung cultured in serum-free chemically defined medium for 48 h. The organ culture technique has been described previously (9, 10). Briefly, 1-mm³ explants of fetal lung were placed on either side of a 60-mm tissue culture dish, containing 2 ml of Waymouth's MB 752/1 medium with 100 U penicillin and 100 μ g streptomycin per ml. The dishes were then rocked from side to side on a rocking platform so that at any one time, half of the explants was exposed to the culture medium and the other half to the atmosphere of 95% O₂-5% CO₂. The explants were exposed to culture medium alone (controls) or medium containing either 100 nM dexamethasone, 100 nM T_3 , or a combination of these two agents for 48 h. These concentrations were shown to be optimal in a previous study (11). At the end of the culture period, explants were harvested for analysis of enzyme activity, glucocorticoid binding, or incorporation of choline into PC and disaturated PC as described previously (9).

Tissue analysis. Phospholipids were extracted in chloroform and methanol and then quantitated by the method of Dodge and Phillips (6). Protein was measured by the method of Lowry *et al.* (20) and DNA by the mithramycin binding technique of Hill and Whatley (14), using bovine serum albumin and calf thymus DNA as standards, respectively.

Glucocorticoid binding. Intact cell binding was determined in 1-mm³ cubes derived from either the *in vivo* or *in vitro* experiments by methods described previously (2, 12). Briefly, the tissue blocks were exposed to 35 nM [³H]dexamethasone in the presence or absence of a 100-fold excess of unlabeled dexamethasone, for 3 h at 37°C. The binding assay reached equilibrium under these conditions. At the end of this incubation, the explants were washed with ice cold saline, homogenized, and separated into a nuclear and cytoplasmic fraction by centrifugation. The nuclear fraction was washed twice to remove unbound dexamethasone, and radioactivity in the nuclear pellet was counted in a scintillation spectrometer. The cytoplasmic fraction was filtered through a column of Sephadex G-25 to separate the bound from the free dexamethasone. The radioactivity in the bound fraction (the eluate) was then determined.

Hormonal levels. Serum total corticosterone concentration was determined by a modification of the competitive protein binding radioassay of Murphy (24). T_3 was determined by a double antibody radioimmunoassay (T_3 kit, Nichols Institute, San Pedro, CA).

Enzyme assays. Fetal lungs were rapidly removed, cleared of nonpulmonary tissue, and homogenates (20%, w/v) were prepared with a Potter-Elvehjem tissue grinder in 0.33 M sucrose/10 mM Tris HCl/1 mM EDTA (pH 7.4). The supernatant fraction was prepared by centrifugation at 105,000 *g* for 1 h. Cholinephosphate cytidylyltransferase was assayed in the supernate and all other enzymes in the homogenate. All assays were optimized with respect to time, protein concentration, pH, and substrate concentrations.

Choline kinase (EC 2.7.1.32) was assayed by measuring the rate of [methyl-¹⁴C]choline incorporation into choline phosphate as described previously (22). Cholinephosphate cytidylyltransfer-

ase (EC 2.7.7.15) was assayed by measuring the rate of incorporation of radioactivity from [methyl-¹⁴C]choline phosphate into CDPcholine as described previously for the rabbit (29) except that the concentration of choline phosphate was 2 mM and that of cytidine triphosphocholine was 8 mM. This enzyme was assayed both with and without the addition of 1.1 mM phosphatidylglycerol to the reaction mixture (29). Cholinephosphotransferase (EC 2.7.8.2) was assayed by measuring the rate of incorporation of radioactivity from CDP [methyl-¹⁴C]choline into PC in the presence of 1,2-dioleoylglycerol or 1,2-dipalmitoylglycerol. The assay with dioleoylglycerol was the same as that described previously (28), except that the concentration of CDPcholine was 0.3 mM. The assay in the presence of dipalmitoylglycerol was the same, except that the diacylglycerol was prepared by sonication at 65°C in the presence of phosphatidylglycerol and Polysorbate 20 as described by Miller and Weinhold (23). When the diacylglycerols were prepared by the latter procedure, cholinephosphotransferase activities were similar in the presence of dioleoylglycerol and dipalmitoylglycerol but, in contrast to the data of Miller and Weinhold (23), were not greater than in the presence of dioleoylglycerol as prepared conventionally (28).

Phosphatidate phosphatase (EC 3.1.3.4) was assayed by measuring the rate of release of inorganic phosphate from phosphatidic acid (3), and lysolecithin acyltransferase (EC 2.3.1.23) by measuring the rate of incorporation of radioactivity from [1-¹⁴C] palmitoylCoA into PC as described previously (9) except that the concentration of palmitoylCoA was 70 μ M and PC was separated by thin layer chromatography in chloroform:methanol:concentrated NH₄OH: water (50:35:4:2, by volume).

Phosphatidate cytidylyltransferase (EC 2.7.7.41) was assayed by measuring the rate of incorporation of [5-³H]CTP into CDPdiacylglycerol and CDPdiacylglycerolinositol-3-phosphatidyltransferase (EC 2.7.8.11) by measuring the rate of incorporation of myo-[2-³H]inositol into phosphatidylinositol as described previously (17). The activities of glycerophosphate phosphatidyltransferase (EC 2.7.8.5) and phosphatidylglycerophosphatase (EC 3.1.3.27) combined were assayed by measuring the rate of incorporation of L-[U-¹⁴C]glycerol-3-phosphate into phosphatidylglycerol as described previously (26) except that 0.4 mM CDPdiacylglycerol from egg PC was used and the pH was 7.4.

Chemicals. Radiochemicals were purchased from New England Nuclear (Boston, MA), CDPdiacylglycerol from Serdary (London, Ontario, Canada) and all other biochemicals from Sigma (St. Louis, MO). Betamethasone (Celestone Soluspan suspension) was obtained from Schering Corporation (Kenilworth, NJ) and dexamethasone and T_3 from Sigma. Culture medium was purchased from Grand Island Biological Co. (Grand Island, NY).

Statistical analysis. In the *in vivo* studies, separate animals were used for each treatment group, whereas in the *in vitro* studies a common pool of explants was prepared from a number of litters and the various treatment groups were then derived from this common pool of tissue. Paired analysis was therefore performed on the *in vitro* material. In all experiments the T_3 and glucocorticoid groups were compared to the control group by two-way analysis of variance. The combined treatment group was evaluated by two-way analysis of variance (for statistical interactions) and by a single *t* test for difference from the glucocorticoid group.

RESULTS

Dose-response relationships for T_3 and betamethasone were established before *in vivo* studies of the effects of combinations of these agents on fetal lung phospholipid synthesis. The effects of varying maternal T_3 injections on fetal serum T_3 levels are shown in Table 1. A small increase occurred with 0.7 mg/kg and a marked increase with 7 mg/kg. An earlier study using the rabbit had shown that high doses of DIMIT, a thyroid hormone analog

which crosses the placenta, produced an increase in cortisol levels in the fetuses (1). For this reason fetal serum corticosterone levels were measured in the rat fetuses. It is apparent that even high doses of T_3 did not produce a rise in total corticosterone levels. The rate of choline incorporation into PC was increased by all the doses of T_3 that were used; because a plateau was reached by 7 mg/kg this dose was used in further studies. A dose-response study with betamethasone was also carried out using doses of 0.2, 0.5, 1.0, and 5.0 mg/kg. Betamethasone was injected into the doe once daily on d 18 and 19 and the rate of choline incorporation into PC was determined in the fetal lungs on d 20. Dose-dependent stimulation was noted with increasing doses up to 1 mg/kg, but there was no further increase above that. One milligram per kilogram of betamethasone was, therefore, used in all further studies.

The effects of injecting T_3 , betamethasone, or a combination of these hormones are shown in Table 2. Injection of T_3 into the mother produced a significant 28-fold increase in fetal serum T_3 levels. Injection of betamethasone resulted in a 2-fold increase in serum T_3 levels and the combination of hormones produced levels which were far in excess of those produced by T_3 alone. Fetal serum corticosterone levels were not increased by T_3 injection and the level of this steroid was below the lower limits of the assay in those animals that had been treated with betamethasone, reflecting suppression of endogenous corticosterone production.

Treatment with T_3 produced a significant, 34% increase in the rate of choline incorporation into PC and betamethasone treatment resulted in a 33% increase, which was also significant. Combination treatment resulted in a 69% increase. This effect was significantly greater than that produced by betamethasone alone. When incorporation into disaturated PC was studied, the effect of T_3 was decreased whereas that of betamethasone was enhanced. The effect of combined treatment was again approximately equal to the sum of the individual effects of these hormones, but was not significantly different from that of betamethasone alone. This is due to the fact that whereas betamethasone produces an increase in the percentage of PC that is disaturated, T_3 does not. In fact T_3 produced a significant decrease in this percentage. This finding is consistent with previous *in vivo* data from this laboratory (11).

When fetal lung PC accumulation was examined (Table 3) it was found that betamethasone produced an increase in the disaturated PC content whereas T_3 did not. Betamethasone treatment again resulted in an increase in the disaturated PC:PC ratio, whereas T_3 treatment did not. This finding is also consistent with *in vitro* observations. Exposure of explants of 18-d fetal rat lung to 100 nM dexamethasone for 48 h resulted in a 2-fold increase in the disaturated PC content but there was no increase after exposure to 100 nM T_3 . These concentrations had been shown previously to produce maximal stimulation of the rate of choline incorporation into PC (11). Similarly, the percentage of PC that was disaturated was increased by dexamethasone, but not by T_3 (data not shown, $n = 3$).

In an attempt to explore possible mechanisms for the enhancement of glucocorticoid action by T_3 , the effect of T_3 on specific cytoplasmic and nuclear binding of dexamethasone was examined *in vivo* and *in vitro* (Table 4). In the *in vivo* studies T_3 (7 mg/kg) was administered for 48 h. T_3 treatment did not result in any change in cytoplasmic or nuclear binding of dexamethasone.

Table 1. Effect of maternal T_3 administration-dose response relationships*

	0†	T_3 injected (mg/kg)		
		0.7	7	70
Fetal serum T_3 (ng/dl)	123‡	175	3350	18,800
Fetal serum corticosterone (ng/ml)	320	295	325	225
Choline incorporated into phosphatidylcholine (nmol · h ⁻¹ · mg ⁻¹ protein)	2.84	3.26	3.57	3.53

* T_3 in saline was injected IM on d 18 and 19 of gestation. The fetuses were examined on d 20.

† Saline was injected into control animals.

‡ Data obtained from one to two experiments.

Table 2. Effect of maternal hormone administration on fetal hormone levels and choline incorporation into phosphatidylcholine*

	Control	T_3	Betamethasone	T_3 + Betamethasone	n†
Fetal serum T_3 (ng/dl)	84 ± 23‡	2380 ± 333	161 ± 50	8550 ± 1955‡	4
Fetal serum corticosterone (ng/ml)	384 ± 22	324 ± 42	<20	<20	5
Choline incorporated into PC (nmol · h ⁻¹ · mg ⁻¹ protein)	2.73 ± 0.14	3.64 ± 0.24	3.62 ± 0.20	4.60 ± 0.29‡	5
% Stimulation		34	33	69	
Choline incorporated into DSPC (nmol · h ⁻¹ · mg ⁻¹ protein)	0.76 ± 0.03	0.92 ± 0.09§	1.17 ± 0.07	1.37 ± 0.10	5
% Stimulation		20	53	79	
DSPC/PC ratio (%)	28.1 ± 0.7	25.0 ± 0.8	32.4 ± 1.0	29.7 ± 0.8	5

* T_3 (7.0 mg/kg) and/or betamethasone (1.0 mg/kg) were injected into mothers on d 18 and 19 and the fetuses examined on d 20. PC, phosphatidylcholine and DSPC, disaturated phosphatidylcholine.

† n, number of experiments.

‡ Data represent the mean ± SE.

§ Statistically significant difference from control value by two-way analysis of variance: $P < 0.05$.

|| $P < 0.005$.

‡ Statistically significant difference from betamethasone value. The combined hormone group was compared only with the betamethasone group by *t* test: $P < 0.05$.

Table 3. Effect of maternal hormone administration on fetal lung phosphatidylcholine content*

	Control	T_3	Betamethasone	T_3 + Betamethasone	n
PC content (μg P/mg protein)	3.53 ± 0.21	3.43 ± 0.10	3.68 ± 0.16	3.60 ± 0.11	5
DSPC content (μg P/mg protein)	0.77 ± 0.06	0.71 ± 0.03	1.00 ± 0.02‡	0.93 ± 0.02	5
DSPC/PC (%)	21.6 ± 0.7	20.7 ± 1.1	27.8 ± 0.7‡	25.9 ± 0.7	5

* Experimental conditions and statistical analysis were as in Table 2. PC, phosphatidylcholine and DSPC, disaturated phosphatidylcholine.

‡ Statistically significant difference from control value, $P < 0.001$.

Explants of 18-d fetal rat lung were also exposed to 100 nM T_3 for 6, 24, or 48 h. Cytoplasmic and nuclear binding of dexamethasone were examined after each of these periods. The only significant finding was that after 48 h there was a small decrease in cytoplasmic binding.

The activities of a number of enzymes of phospholipid synthesis were also measured in the lungs of fetuses that had been treated *in vivo* (Table 5). The only significant finding was that both betamethasone and T_3 significantly increased the activity of cholinephosphate cytidyltransferase when this enzyme was assayed in the absence of phosphatidylglycerol. Treatment with T_3 resulted in a 43% increase in activity and with betamethasone, a 96% increase. Combined hormone treatment produced a 166% increase. Although the effect of the combined hormones was equal to the arithmetic sum of the effects of the individual

hormones, it was not statistically significantly different from the effect of betamethasone alone. Addition of phosphatidylglycerol to the assay mixture resulted in a marked increase in the activity of the enzyme in all experimental groups and abolished the effect of hormone treatment.

Similar data were obtained in the *in vitro* experiments. The activities of all the enzymes listed in Table 5 were examined after exposure of explants of 18-d lung to dexamethasone (100 nM), T_3 (100 nM), or a combination of the two hormones for 48 h. As is shown in Table 6, when cholinephosphate cytidyltransferase was assayed without phosphatidylglycerol there was a 56% increase after exposure to T_3 , a 99% increase after exposure to dexamethasone, and a 158% increase after exposure to both hormones. Although the combined hormone effect was again equal to the arithmetic sum of the effects of the two hormones, it was also not significantly different from the dexamethasone effect. The activity of none of the other enzymes examined was significantly increased by exposure to either hormone *in vitro*. There was no significant difference in protein:DNA ratio after injection of any of the hormones alone or in combination.

Table 4. Effect of exposure to T_3 *in vivo* and *in vitro* on glucocorticoid binding*

	Control	T_3
<i>In vivo</i> †		
Cytoplasmic binding	112 ± 15‡	84 ± 7
Nuclear binding	233 ± 23	236 ± 17
<i>In vitro</i> §		
Cytoplasmic binding		
6 h	76 ± 5	73 ± 7
24 h	107 ± 16	99 ± 17
48 h	138 ± 4	106 ± 3
Nuclear binding		
6 h	196 ± 5	212 ± 10
24 h	274 ± 12	264 ± 15
48 h	308 ± 27	274 ± 39

* Binding was determined by exposing 1-mm³ blocks of lung tissue to 35 nM [³H]dexamethasone for 3 h in the presence or absence of 5 μ M unlabeled dexamethasone.

† T_3 was administered to the mother as described in footnote (asterisk) of Table 2.

‡ Data are expressed as fmol dexamethasone specifically bound/mg protein and represent the mean ± SE of three to four experiments.

§ Explants of 18-d fetal lung were exposed to 100 nM T_3 for the period indicated.

|| Statistically significant difference from control value by *t* test; *P* < 0.001.

DISCUSSION

Recent clinical studies of dexamethasone administration for the prevention of RDS in the newborn have suggested that glucocorticoids might not be as effective for this purpose as was previously believed (4). With the publication of favorable developmental follow-up studies of infants treated antenatally with glucocorticoids (21), concern regarding the use of these hormones has turned from the issue of toxicity to that of effectiveness. As a first step in exploring the possible role of combined hormone

Table 6. Effect of hormones on cholinephosphate cytidyltransferase activity*

Control	T_3	Dexamethasone	T_3 + Dexamethasone
0.77 ± 0.07†	1.20 ± 0.14‡	1.53 ± 0.23‡	1.99 ± 0.43

* Explants of 18-d fetal rat lung were cultured for 48 h. Concentrations of both dexamethasone and T_3 were 100 nM.

† Data represent the mean ± SE of eight experiments and are expressed as nmol · min⁻¹ · mg protein⁻¹.

‡ Statistically significant difference from control value, *P* < 0.05. Statistical analysis was by repeated measures analysis of variance.

Table 5. Effect of maternal hormone administration on fetal lung enzyme activities*

Enzyme	Control	T_3	Betamethasone	T_3 + Betamethasone	<i>n</i>
Phosphatidate cytidyltransferase	2.53 ± 0.19**	2.69 ± 0.20	2.72 ± 0.10	2.79 ± 0.12	4
Phosphatidate phosphatase	8.83 ± 1.03	7.87 ± 0.18	9.54 ± 0.52	9.34 ± 0.54	4
Choline kinase	1.06 ± 0.08	1.07 ± 0.10	1.19 ± 0.11	1.16 ± 0.08	4
Cholinephosphate cytidyltransferase					
-PG†	0.82 ± 0.05	1.17 ± 0.08†	1.61 ± 0.20#	2.18 ± 0.32	4
+PG‡	4.89 ± 0.32	5.41 ± 0.28	5.77 ± 0.29	5.64 ± 0.09	4
Cholinephosphotransferase					
+dioleoylglycerol§	1.36 ± 0.23	1.65 ± 0.08	1.61 ± 0.15	1.56 ± 0.15	4
+dipalmitoylglycerol	1.06 ± 0.13	1.21 ± 0.08	1.29 ± 0.07	1.13 ± 0.07	4
Lysolecithin acyltransferase	1.66 ± 0.20	1.89 ± 0.24	1.73 ± 0.19	1.92 ± 0.15	4
Glycerophosphate phosphatidyltransferase + phosphatidylglycerophosphatase	0.076 ± 0.004	0.078 ± 0.003	0.071 ± 0.004	0.082 ± 0.005	4
CDP diacylglycerol-inositol 3-phosphatidyltransferase	1.46 ± 0.11	1.55 ± 0.10	1.69 ± 0.09	1.65 ± 0.10	4

* Experimental conditions and statistical analysis are described in Table 2.

** Enzyme activity expressed as nmol · min⁻¹ · mg protein⁻¹.

† Assayed without phosphatidylglycerol in the reaction mixture.

‡ Assayed with phosphatidylglycerol in the reaction mixture.

§ Assayed with dioleoylglycerol as substrate.

|| Assayed with dipalmitoylglycerol as substrate.

† Statistically significant difference from control value *P* < 0.05.

P < 0.01.

administration in the prevention of RDS, we had shown previously that there were additive interactions between T_3 and dexamethasone with regard to some parameters of PC synthesis in organ cultures of fetal rat lung (11).

In order to examine this role *in vivo*, we felt that it was desirable to develop a model in which the effect of T_3 on the fetus could be examined after maternal (as opposed to fetal) injection. In most species thyroid hormones do not cross the placenta to any great extent and for this reason studies of thyroid effects on fetal lung maturation had usually involved direct fetal injection, usually of thyroxine (T_4). Fetal injection, even of saline, is a stress which in and of itself results in accelerated lung maturation (27), thereby complicating the interpretation of the findings. Knobil and Josimovich (18) showed that there was significant transplacental passage of T_3 and T_4 in the rat. As our data in this study indicate, it is possible to obtain a serum level of T_3 that produces maximal stimulation of choline incorporation into PC with a maternal injection of $7 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$. The resulting mean fetal serum level (24 h after the last injection) of 2380 ng/dl is equivalent to a concentration of 37 nM. Even if a large amount of the hormone is bound, this concentration is still well above the level at which the dose-response curve for T_3 stimulation of choline incorporation into PC plateaued in our organ culture studies (11), which is consistent with the finding that higher doses of T_3 produced no further stimulation of choline incorporation. It is also of interest that, unlike DIMIT administration to the pregnant rabbit (1), T_3 injection did not produce elevated fetal levels of circulating glucocorticoid.

Betamethasone treatment resulted in a significant increase in fetal T_3 levels. It is known that glucocorticoids promote the conversion of fetal T_4 and T_3 , rather than reverse T_3 (19) and this could account for the increase in T_3 levels. The tremendous increase in T_3 levels produced by combined hormone administration could only have come about by increased passage of hormone across the placenta. Analysis of variance indicated that betamethasone and T_3 had synergistic effects with regard to fetal T_3 levels.

Both betamethasone and T_3 significantly increased the rate of choline incorporation into PC. In the case of the combined hormone treatment the effect was equal to the sum of the individual hormone actions, an additive effect. The combined effect was significantly different from the betamethasone effect when a single *t* test was applied to these two results. T_3 did not, however, produce an increase in DSPC content, whereas betamethasone did. The reason for this is not clear. Ballard *et al.* (1), using DIMIT in the fetal rabbit, had shown that there was a marked increase in lung lavage PC content. Because we were measuring whole lung disaturated PC content (a good lavage procedure cannot be performed in the fetal rat), it is possible that our measurements were not sensitive enough to detect an increase in surfactant-related disaturated PC. An alternative explanation is that T_3 treatment may result in increased disaturated PC turnover, with no accumulation of this phospholipid in the lung tissues.

The betamethasone effect on choline incorporation could not be accounted for by the increase in T_3 levels because the two hormones produce different effects *in vivo* and *in vitro* (10). Similarly the combined hormone effect could not be accounted for by the large increase in T_3 levels as the dose-response data indicated that administration of $7 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ of T_3 alone resulted in the maximal T_3 effect.

Our findings are, in general, consistent with those of Hitchcock (15) who found that metopirone treatment or adrenalectomy decreased the effect of T_4 (injected directly into rat fetuses) on the morphologic maturation of the lung. Dow and Torday (7) have also indicated in a preliminary report that whereas direct injection of T_4 into fetal rabbits produced no greater stimulation of choline incorporation into disaturated PC than did saline, T_4 injection did enhance the effect of directly injected dexamethasone.

The enhancement of glucocorticoid action by T_3 could not be accounted for by any change in cytoplasmic or nuclear binding of the steroid. This finding is similar to that reported in pituitary cells in which there are interactions between T_3 and glucocorticoid with regard to growth hormone synthesis (31). In that system T_3 did not produce any change in glucocorticoid binding or vice versa. The work of Smith and Sabry (33) suggests that T_3 increases the sensitivity of the type II cell to fibroblast pneumocyte factor, which is believed to be released by mesenchymal cells in response to glucocorticoid stimulation.

Fetal lung cholinephosphate cytidyltransferase activity has previously been reported to be stimulated by glucocorticoids and estrogen in a number of species, both *in vivo* and *in vitro* (3, 5, 10, 17, 29, 30). To our knowledge this is the first report of stimulation of this enzyme by T_3 . Fetal lung choline-phosphate cytidyltransferase activity is increased by inclusion of acidic phospholipids in the reaction mixture (8, 16). In this and previous studies (16, 17) the stimulatory effects of the hormones were not observed when the enzyme was assayed in the presence of phosphatidylglycerol. This suggests that the hormones produce their effects by activating the enzyme rather than by increasing its amount. There is accumulating evidence from nonpulmonary systems that cholinephosphate cytidyltransferase catalyzes the rate-limiting step in PC biosynthesis (32, 34–37) and in a recent study, Post *et al.* (25) showed that is also the rate-limiting step in PC synthesis in type II cells isolated from adult rat lung. It remains, however, to be established if these hormonal effects on enzyme activity are of physiologic significance. Although only cholinephosphate cytidyltransferase was stimulated by glucocorticoids in this study, other enzymes of lung phospholipid synthesis (particularly phosphatidate phosphatase in the rabbit) have also been reported to be stimulated by these steroids in other studies (30).

The data reported here, therefore, suggest that there is an additive interaction between T_3 and betamethasone with regard to choline incorporation into PC. This supports the view that combined hormone administration may be more effective than single hormones in preventing RDS in humans. More information is, however, clearly required before clinical use of combined hormone therapy can be considered.

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