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Pre-translational regulation of lipid synthesizing enzymes and surfactant proteins in fetal rat lung in explant culture

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In hormone-free explant cultures of 18-day fetal rat lung the levels of the mRNAs for fatty acid synthase, ATP citrate lyase and surfactant proteins A, B, and C, increased as they do in vivo. Also CTP:phosphocholine cytidylyltransferase mRNA increased spontaneously. Unlike in vivo, malic enzyme mRNA increased drastically in cultured explants. Culture with dexamethasone increased the abundance of fatty acid synthase and surfactant protein mRNAs, but considerably depressed that of malic enzyme mRNA. Dexamethasone had little effect on CTP:phosphocholine cytidylyltransferase mRNA, supporting the concept that the increased activity of this enzyme caused by glucocorticoid is due to increased fatty acid synthesis.

Lung surfactant, Glucocorticoid; Lipogenic enzyme; Fatty acid synthase; CTP:phosphocholine cytidylyltransferase; Surfactant protein

1. INTRODUCTION

Pulmonary surfactant, a surface-active material preventing alveolar collapse and transudation, is produced by the type II alveolar epithelial cells [1]. It contains 70–80% phospholipids and 5–10% proteins. Dipalmitoylphosphatidylcholine is the major phospholipid component [1]. Four surfactant proteins have been described: the hydrophilic glycoproteins, SP-A and SP-D, and the hydrophobic proteins, SP-B and SP-C [2]. The synthesis of the surfactant in fetal type II cells starts in the terminal part of gestation and dramatically accelerates in the late prenatal period [3]. Results from many studies indicate that endogenous glucocorticoids are involved in the acceleration of surfactant synthesis [4]. In accordance with this idea, exposure of fetal lung in vivo or in explant culture to glucocorticoid leads to increased synthesis of surfactant components [2,4,5]. In lung explant cultures, glucocorticoid increases the activity of fatty acid synthesis de novo [6] and of the enzymes fatty acid synthase (FAS) [6,7] and CTP:phosphocholine cytidylyltransferase (CPCT) [8,9]. The latter catalyzes a rate-regulatory step in phosphatidylcholine formation. Despite the large body of evidence demonstrating that glucocorticoids can accelerate the formation of surfactant components, results from several studies have indicated that this acceleration also occurs in the absence of glucocorticoids and in the presense of the glucocorticoid antagonist, RU 486; the glucocorticoids appear to exert a modulating, rather than an initiating, effect on lung maturation [4].

In the present investigation, explants of fetal rat lung tissue were cultured in serum-free medium. A characteristic of this culture system, in which it differs from culture systems involving isolated cells, is that native cell-to-cell interactions are maintained. The first goal was to study the spontaneous development, which is influenced by factors endogenous to the lung, of the mRNAs coding for CPCT and for FAS, and three other enzymes involved in fatty acid synthesis, acetyl-CoA carboxylase (ACC), ATP citrate lyase (ACL) and malic enzyme (ME). The second goal was to investigate the additional effect of dexamethasone on the levels of the various mRNAs. The third goal was to compare the spontaneous and dexamethasone-induced changes in the abundance of the mRNAs of these lipid-synthesizing enzymes with those occurring in the levels of the mRNAs coding for surfactant proteins.

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2. MATERIALS AND METHODS

2.1. *Materials*

The following plasmids were used to make probes for Northern blot hybridization analysis: clone p18.1-6 (0.5 kb insert encoding rat mammary gland ACC), clone pFAS-5 (1.1 kb insert encoding mouse liver FAS [10]), clone pACL2 (1.5 kb insert encoding mouse liver ACL [11]), clone pR ME1 (2.4 kb insert encoding rat liver malic enzyme [12]), a clone containing a 1.3 kb insert encoding rat liver CPCT [13], clone pHFβA-3'UT-HF (0.4 kb insert encoding human β-cytoplasmic actin [14]), a clone containing a 662-bp insert encoding rat lung SP-A [15], clone SP-B 4-3 (1.8 kb insert encoding human SP-B [16] and clone RJ2-1 (0.8 kb insert encoding human SP-C [17]. [α-^{32}P]dATP (3,000 Ci/mmol) was obtained from New England Nuclear (Wilmington, DE). Pregnant Wistar rats of known gestation time (term is at day 22)

were obtained from the Central Animal Laboratory, Utrecht University.

2.2. *Fetal lung explant culture*

Fetal rat lung explants were cultured as described by Gross et al. [18]. Briefly, lungs obtained under sterile conditions from rat fetuses at day 18 of gestation were chopped into 1-mm^3 cubes. The cubes were placed in 60-mm tissue culture dishes containing 2 ml serum-free Waymouth MB 752/1 medium (Gibco, Paisley, Scotland) containing penicillin (100 U/ml) and streptomycin (100 μg/ml), and were incubated on a rocking platform under an atmosphere of 5% CO_2 /95% air at 37°C for 48 h in the presence or absence of 100 nM dexamethasone.

2.3. *Isolation and Northern blot hybridization of RNA*

At the end of the culture period, the explants were scraped from the dishes with a rubber policeman, rinsed twice with 0.9% NaCl, quickly frozen in liquid nitrogen and stored at −70°C. From this material the RNA was isolated by the guanidine isothiocyanate/CsCl method [19]. RNA was quantified by measuring the absorbance at 260 nm. Aliquots containing 30 μg total RNA were resolved by electrophoresis through a 1.2% agarose-formaldehyde gel [20]. After the separation the RNA was transferred to nylon membrane (Nytran-N, Schleicher and Schuell, 's-Hertogenbosch, The Netherlands) by capillary blotting. Staining with Methylene blue showed that equal amounts of RNA were present on the blot in each lane. The blots were heated at 80°C for 2 h. They were prehybridized for 3 h and subsequently hybridized for 16–18 h as described [21] using 0.25 μg ^{32}P-labeled cDNA. The ^{32}P-labeled probes (approx. 4×10^8 cpm/μg DNA) were prepared by random priming [22] from [α-^{32}P]dATP and cDNA inserts that had been excised from the plasmids with the appropriate restriction enzymes. After hybridization the blots were washed [21] and autoradiograms of the blots were made at −80°C. The intensities of the hybridization signals on the autoradiograms were quantified by densitometry, using an LKB Ultroscan XL laser densitometer.

RESULTS

3.1. *Abundance of the mRNAs encoding lipid-synthesizing enzymes and surfactant proteins as a function of culture time*

In order to study whether, in the absence of exogenous hormones, fetal lung tissue maintained in explant culture was able to pursue its normal differentiation process as far as changes in the mRNAs for FAS, ACC, ACL, ME, CPCT and surfactant proteins was concerned, we measured the abundance of the various mRNAs in lung explants after different culture times. Fig. 1 shows that the content of mRNA encoding FAS and ME increased drastically in culture, to 600% and 500%, respectively, after 48 h. For ME mRNA the increase was already significant after 4 h of culture. The CPCT mRNAs also increased in abundance, although

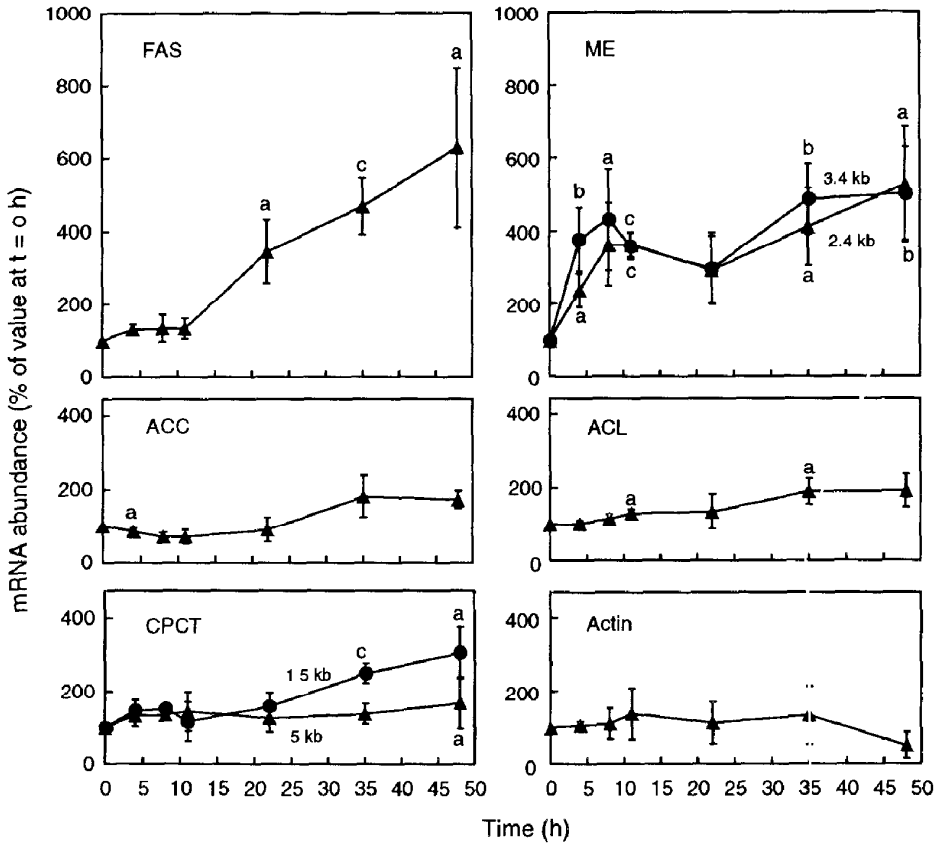

Fig. 1. Relative abundance of mRNAs encoding lipid synthesizing enzymes and β-actin in fetal rat lung explants as a function of culture time. For each experiment the abundance of a particular mRNA was expressed as a percentage of the abundance at t = 0 h. Data represent means ± S.E.M. of 4 independent experiments. Significantly different from value at t = 0 h according to Student's *t*-test: $^a 0.025 < P < 0.05$, $^b 0.01 < P < 0.025$, $^c P < 0.01$.

to a lesser extent. After 48 h of culture the 1.5 kb and the 5 kb CPCT mRNA reached levels of 300% and 150%, respectively. The levels of ACC and ACL mRNAs increased nearly 2-fold during 35 h of culture, although this increase was only statistically significant for ACL mRNA. SP-A, SP-B and SP-C mRNAs increased considerably during the 48 h culture period (Fig. 2). After 48 h of culture the SP-B and SP-C mRNAs had increased to 600%, while SP-A mRNA had reached 2,500% of the original abundance.

The mRNA encoding β-actin was used as an internal control. Its level remained more or less constant during culture time (Fig. 1). This indicates that the increases seen for the other mRNAs are not due to any non-specific effect on mRNAs in general.

3.2. *Effect of dexamethasone on the abundance of the mRNAs as a function of culture time*

The effect of dexamethasone on the abundance of the various mRNAs was examined as a function of culture time. For this purpose explants were exposed to 100 nM dexamethasone from the onset of the culture period. Previous experiments [6,7] had shown that 100 nM dexamethasone optimally increased the activity of FAS in fetal lung explants. At regular intervals explants were harvested and the mRNA content was compared with that in explants that had been cultured in parallel in the absence of dexamethasone. Fig. 3 shows that the abundance of FAS mRNA was increased significantly by dexamethasone during the first 16 h of culture. At 11 h of culture a maximal glucocorticoid effect (2.5-fold increase) was observed. The glucocorticoid effect decreased at later time points. The observation of a dexamethasone-induced increase of FAS mRNA is in agreement with an abstract by Palayoor et al. [23], however, that group observed the effect after 48 h of culture (the only time point studied), while in our experiments the glucocorticoid effect was only apparent during the first 16 h (Fig. 3). We have no explanation for this difference. Contrary to the effect on FAS mRNA, dexamethasone caused a marked decrease of the 3.4 and 2.4 kb ME mRNAs. This decrease by glucocorticoid was already significant after 8 h of culture, while after 48 h the abundance of ME mRNA in dexamethasone-treated explants was only 20% of that in control explants. The mRNAs of CPCT were only slightly influenced by dexamethasone exposure, although the 5 kb mRNA was significantly increased at 16 h and the 1.5 kb mRNA was significantly depressed at 48 h. Dexamethasone initially had little effect on ACL mRNA, but after 24 h a decrease was observed (significant at 24 h and 48 h).

The effect of dexamethasone on SP-A mRNAs as a function of culture time was similar to that on FAS mRNA (Fig. 4). Dexamethasone caused an increase in SP-A mRNA levels during the first 16 h of culture. At 11 h a maximal 10-fold stimulation was found for the 1 kb SP-A mRNA. Like for FAS mRNA, the degree of glucocorticoid enhancement of SP-A mRNA levels decreased at longer culture times. A similar profile was observed for SP-C mRNA, although the magnitude of the dexamethasone stimulation was smaller (maximally about 2.5-fold) and the variability among experiments was larger. For SP-B mRNA there was a significant increase by dexamethasone after 16 h of exposure. The maximal stimulation (2-fold) was reached after 24 h and was maintained up to 48 h.

Fig. 3 shows that dexamethasone had no significant effect on the level of actin mRNA at any time during culture, which indicates that the dexamethasone-induced changes seen for the other mRNAs are not due to non-specific effects on mRNAs in general.

4. DISCUSSION

In vivo, FAS mRNA in fetal rat lung increases 3-fold

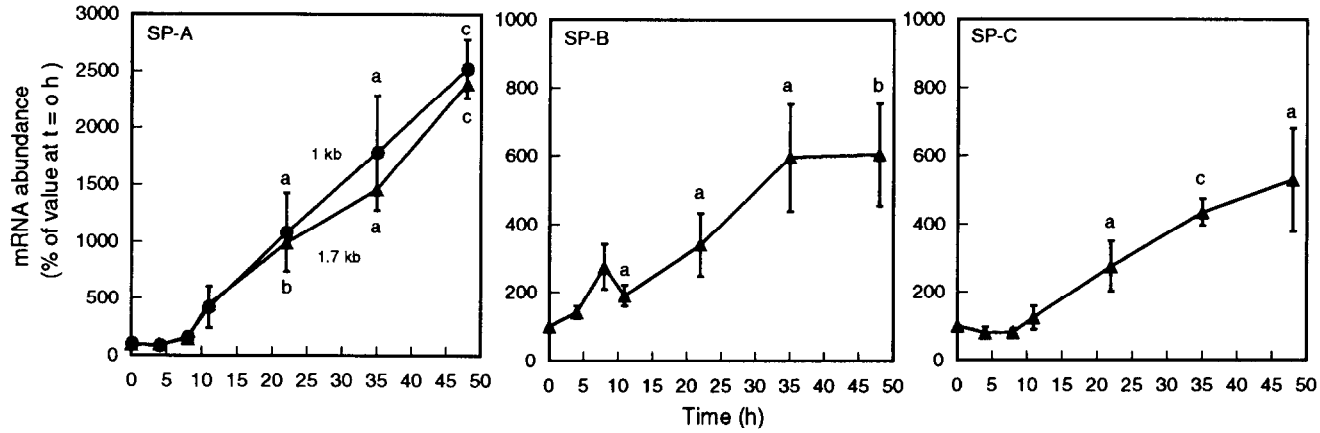

Fig. 2. Relative abundance of mRNAs encoding surfactant proteins in fetal rat lung explants as a function of culture time. For each experiment the abundance of a particular mRNA was expressed as a percentage of the abundance at t = 0 h. Data represent means ± S.E.M. of 4 independent experiments. Significantly different from value at t = 0 h according to Student's *t*-test: $^{a}0.025 < P < 0.05$, $^{b}0.01 < P < 0.025$, $^{c}P < 0.01$.

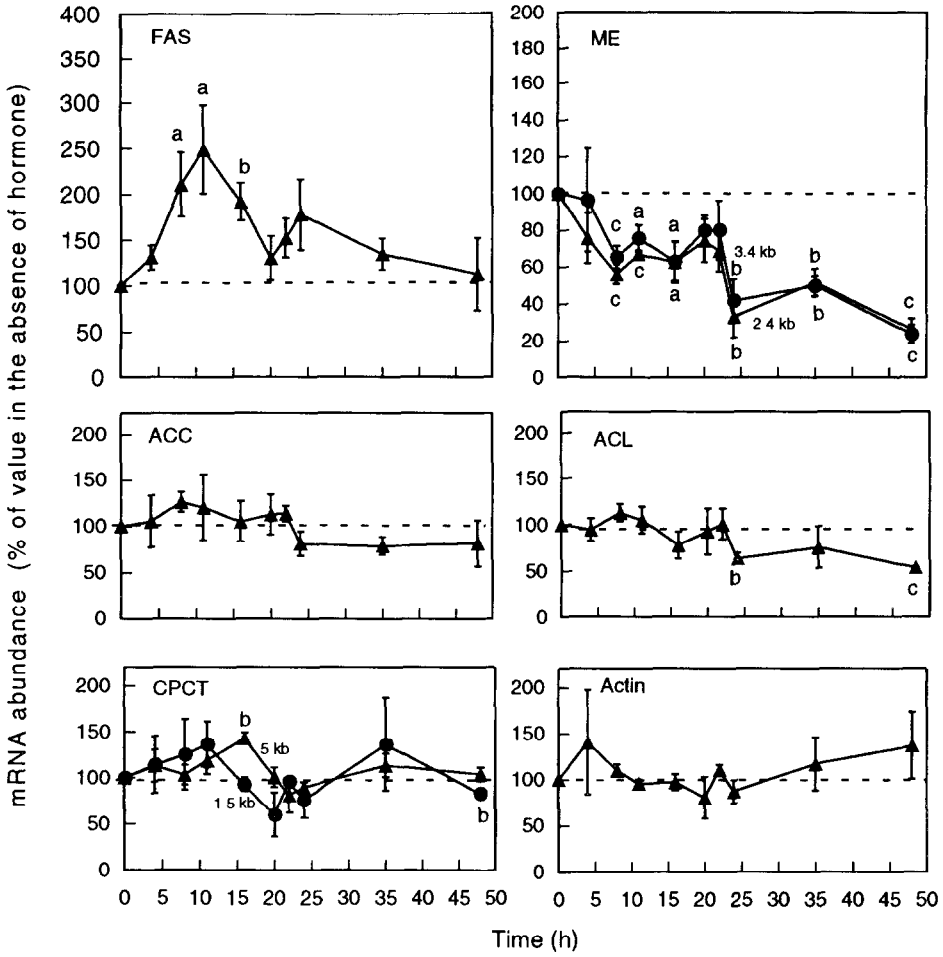

Fig. 3. Effect of dexamethasone on the abundance of mRNAs encoding lipid synthesizing enzymes and β-actin in fetal rat lung explants as a function of culture time. The abundance of a particular mRNA in explants cultured in the presence of dexamethasone is expressed as a percentage of the abundance in control explants that had been cultured for the same length of time in the absence of the hormone. Data in the figure represent means ± S.E M. of 3 independent experiments. Significantly different from no-hormone control according to Student's t-test: $^{a}0.025 < P < 0.05$, $^{b}0.01 < P < 0.025$, $^{c}P < 0.01$. The dotted line indicates the control level.

between days 18 and 21 [21]. The present study shows that in explant culture in the absence of hormones the abundance of FAS mRNA increases as it does in vivo (Fig. 1). This indicates that factors endogenous to the lung can initiate the increase of FAS expression; however, the observation that, in addition, dexamethasone increases FAS mRNA (Fig. 3) indicates that besides endogenous factors, the prenatal surge in glucocorticoids may also be involved in this regulation.

In fetal rat lung in vivo ME mRNAs do not change between days 18 and 22 [21]. Our present results show that, by contrast, in explant culture in the absence of hormones, ME mRNAs increased considerably in 48 h (Fig. 1). This could mean that in vivo the level of ME mRNAs, which tends to be increased by factors endogenous to the lung, is kept in check by a negative hormonal control. As we observed that dexamethasone could strongly depress the ME mRNA level in the explant culture (Fig. 3), it is conceivable that in vivo glucocorticoid acts as a suppressor of the ME mRNA increase. For ACL and ACC mRNA the pattern in explant culture without hormone (Fig. 1) is very similar to the pattern in the fetal lung in vivo [21]. Recently we found that the levels of the mRNAs encoding FAS, ACC and ACL in fetal type II cells were increased approximately 2-fold by glucocorticoid (via interaction with fibroblasts), while ME mRNA remained unchanged [24]. Although in the present experiments with explants FAS mRNA was induced to the same degree, this was not the case for the mRNAs of ACC and ACL. This might suggest that in the explant system the enhancing effect of glucocorticoid on ACC and ACL mRNA in type II cells is suppressed.

In the late prenatal period there is an increase in the activity of CPCT in fetal rat lung [3]. The mechanism of the increased activity is not yet clear [3]. Glucocorticoid exposure increases CPCT activity but not CPCT amount in fetal lung explants [8]. It has been suggested

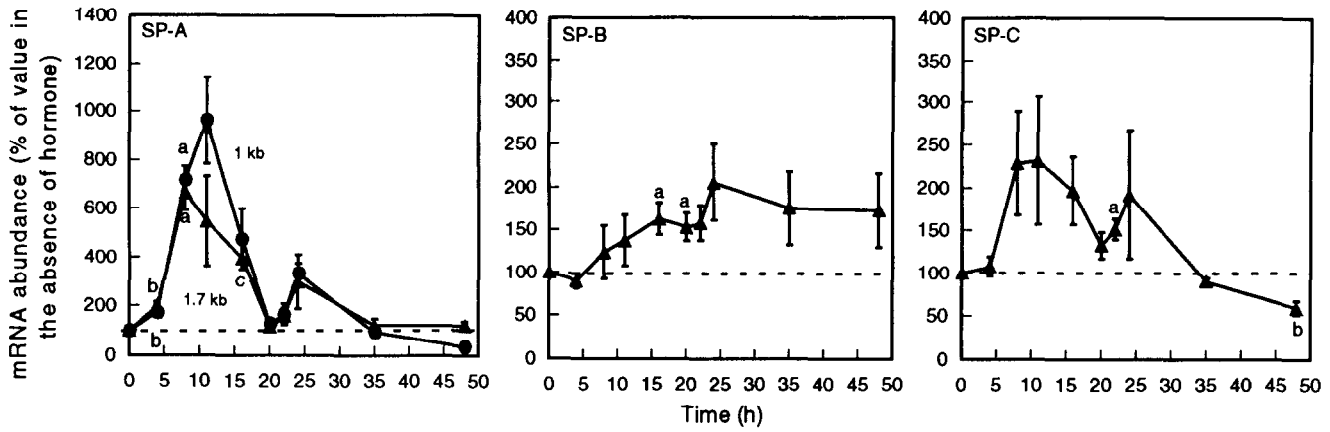

Fig. 4. Effect of dexamethasone on the abundance of mRNAs encoding surfactant proteins in fetal rat lung explants as a function of culture time. The abundance of a particular mRNA in explants cultured in the presence of dexamethasone is expressed as a percentage of the abundance in control explants that had been cultured for the same length of time in the absence of the hormone. Data in the figure represent means ± S.E.M. of 3 independent experiments. Significantly different from no-hormone control according to Student's t-test: $^{a}0.025 < P < 0.05$, $^{b}0.01 < P < 0.025$, $^{c}P < 0.01$. The dotted line indicates the control level.

that the increase in CPCT activity is secondary to the induction of fatty acid synthesis by glucocorticoid [8,9]. Until now neither the developmental pattern of CPCT mRNA in fetal lung in vivo or in explant culture, nor the effect of glucocorticoid on CPCT mRNA in fetal lung, has been reported. In the present study we observed a spontaneous increase of CPCT mRNA in cultured explants (Fig. 1). This could indicate that in vivo there is also an increase of CPCT mRNA from day 18 onward. This idea, however, will have to be corroborated by studies in vivo. Glucocorticoid had at best a small increasing effect on CPCT mRNA in the explants (Fig. 3). A small (fibroblast-mediated) increase of CPCT mRNA by glucocorticoid was recently also observed in fetal type II cells [24].

Interestingly, both the spontaneous increase in the absence of hormone and the stimulation by dexamethasone show the same pattern for FAS mRNA and for SP-A and -C mRNAs (Figs. 1–4). As for FAS mRNA, glucocorticoid increased SP-A and -C mRNAs at the beginning of the culture period, when the spontaneous increase of mRNA had not yet started. The similarity in patterns for FAS mRNA and for the surfactant protein mRNAs might reflect a common regulatory mechanism. The spontaneous increase in the mRNAs of the three tested surfactant proteins is very similar to the pattern observed in fetal rat lung in vivo [25]. A spontaneous increase of SP-A mRNA in cultured fetal rat lung explants has been reported earlier [26]. To our knowledge, such a spontaneous increase in the mRNAs for SP-B and SP-C in explant cultures of fetal rat lung has not been reported before. For SP-C mRNA in cultured human lung explants both a small spontaneous increase [2] and a 17-fold spontaneous decrease [27] have been reported. Our observation that glucocorticoid increases SP-A, -B and -C mRNA in fetal rat lung explants agrees with earlier reports [28–30], however, detailed comparison of our data concerning the glucocorticoid effects with the earlier reports is difficult due to differences in experimental design. In the first place, the earlier studies [28–30] involved culture of the explants in an atmosphere of 95% O_2 It is known that oxygen exposure in vivo leads to increased levels of the mRNAs of SP-A, -B and -C [31]. Secondly, in the earlier studies [28–30] glucocorticoid was not added at the onset of the culture experiment but only after various preincubation periods in hormone-free medium.

In summary, the present paper shows that in fetal rat lung in explant culture FAS, ACC, ACL, ME, CPCT and SP-A, -B and -C are all regulated at a pre-translational level, but in different ways. Of the mRNAs for lipid synthesizing enzymes, FAS and ME mRNA show the most pronounced rise in the absence of hormone, while increases in ACC, ACL and CPCT mRNA are more modest. Like the mRNAs for the surfactant proteins, FAS mRNA is considerably increased by dexamethasone exposure, while ME mRNA is severely depressed. Glucocorticoid has only a small increasing effect on CPCT mRNA. The small size of this latter effect supports the idea [8,9] that pre-translational regulation of the CPCT gene itself is not primarily responsible for the increase in CPCT activity observed in fetal lung tissue upon glucocorticoid treatment.

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