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Pre-translational regulation by glucocorticoid of fatty acid and phosphatidylcholine synthesis in type II cells from fetal rat lung

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Exposure to fibroblast-conditioned cortisol-containing medium increased fatty acid synthase activity and fatty acid synthase, acetyl-CoA carboxylase and ATP citrate lyase mRNA abundance in fetal type II alveolar epithelial cells. Both fibroblast conditioning and cortisol in the medium were required for maximal effect on the mRNA levels, indicating involvement of mesenchymal–epithelial interaction in the cortisol effects. The observed effects provide evidence for an earlier hypothesis that increased activity of CTP:phosphocholine cytidylyltransferase in lung tissue caused by glucocorticoid is due to increased fatty acid synthesis. However, evidence suggesting pre-translational regulation of this enzyme by glucocorticoid was also found.

Lung surfactant; Fatty acid synthase; Acetyl-CoA carboxylase; ATP citrate lyase; CTP:phosphocholine cytidylyltransferase; Glucocorticoid

1. INTRODUCTION

Pulmonary surfactant, the surface-active material that prevents alveolar collapse and transudation, consists of approximately 90% lipid and 10% protein [1]. Dipalmitoylphosphatidylcholine is the major active component [1]. The surfactant is produced by the type II alveolar epithelial cells [2,3]. Its formation in the fetal lung is greatly accelerated towards the end of gestation [4,5] and endogenous glucocorticoids are involved in this process [6,7]. The effect of glucocorticoids on the acceleration of surfactant lipid synthesis is dependent on mesenchymal–epithelial interaction. This was concluded from studies with type II cells and fibroblasts isolated from fetal lung showing that the stimulation by glucocorticoid of the synthesis in type II cells of disaturated phosphatidylcholine (DSPC), which is mainly dipalmitoylphosphatidylcholine, is mediated by a fibroblast-pneumonocyte factor (FPF) that is produced by lung fibroblasts under the influence of glucocorticoid [8–11]. Fetal rat type II cells exposed to fibroblast-conditioned, cortisol-containing medium showed an incorporation of [Me-^{3}H]choline into DSPC that was 179% of the value seen after exposure to control medium [9]. As in other cells, CTP:phosphocholine cytidylyltransferase (CPCT) catalyzes a rate-regulatory step in phosphatidylcholine formation by type II cells [12,13]. Maternal administration of dexamethasone leads to an increased activity of CPCT in type II cells isolated from rat fetuses [14]. In fetal rat type II cells exposed to fibroblast-conditioned, cortisol-containing medium the CPCT activity was increased 2-fold compared to control medium [11].

The activities of fatty acid synthase (FAS) [15,16] and acetyl-CoA carboxylase (ACC) [16,17] and the rate of fatty acid synthesis [15,17] in fetal rat lung show a peak in the period of accelerated surfactant formation. In this period there is also a peak (around fetal day 21; with an increase of 70–100% between fetal days 19 and 21) in the abundance of the mRNAs of FAS, ACC and ATP citrate lyase (ACL) in fetal rat lung [18], indicating pre-translational regulation of these enzymes in the perinatal period. FAS activity in fetal lung tissue is increased by glucocorticoids, both in vivo [15] and in explant culture [19–21]. The increased FAS activity in lung explant culture is due to induction of this enzyme [19], which is brought about at a pre-translational level [22].

Recently, it was observed that glucocorticoid increased the activity but not the amount of CPCT in explants of fetal rat lungs [23] and that inhibition of fatty acid synthesis abolished the stimulatory effect of glucocorticoid on CPCT in these lungs [20]. Based on these findings and earlier observations that the activity of CPCT in lung can be stimulated by fatty acids and various phospholipids [24,25], Rooney and collaborators [23,26] hypothesized that the stimulatory effect of glucocorticoids on CPCT activity in fetal lung is the result of increased synthesis of fatty acids which, either as free fatty acids or after incorporation into phospholipids, activate CPCT.

We have previously shown that exposure of type II cells isolated from fetal rat lung to fibroblast-conditioned, cortisol-containing medium leads to an enhancement of fatty acid synthesis in these cells [16]. The pres-

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ent study was undertaken to investigate whether, under the same conditions, the activity of FAS is increased and if so, whether this is brought about at a pre-translational level. Moreover, it was studied whether there is pre-translational regulation of other enzymes involved in fatty acid synthesis and of CPCT. In addition, we asked ourselves the question whether fibroblast-type II cell interaction is required for the effects.

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 [27]), clone pACL2 (1.5 kb insert encoding mouse liver ACL [28]), clone pR ME1 (2.4 kb insert encoding rat liver malic enzyme [29]), a clone containing a 1.3 kb insert encoding rat liver CPCT [30], clone pHFβA-3'UT-HF (0.4 kb insert encoding human β-cytoplasmic actin [31]) and clone pHcGAP (1.2 kb insert encoding human liver glyceraldehyde-3-phosphate dehydrogenase [32]. [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 purchased from Harlan-CPB in Zeist, The Netherlands.

2.2. *Cell isolation and incubation*

Type II cells and fibroblasts were isolated from 19-day fetal rat lungs by differential adherence, as described before [33].

Conditioned media were prepared as described by Post and Smith [10] in an article demonstrating the involvement of type II cell-fibroblast interaction in the stimulation by cortisol of phosphatidylcholine synthesis in type II cells. Fibroblasts, that had attached to T-flasks during the first 1-h attachment period [33] in minimal essential medium (MEM) containing 10% fetal bovine serum were rinsed with serum-free MEM and grown to confluence in MEM containing 10% charcoal-stripped newborn calf serum. At confluence, fibroblasts were rinsed with serum-free MEM and subsequently incubated for 48 h in either serum-free MEM containing 10^{-7} M cortisol or MEM without cortisol. After the exposure the medium was collected, centrifuged to remove any detached cells, incubated for 2 h at 58°C [8,10] and stored at -20°C. Portions of the MEM and MEM containing 10^{-7} M cortisol that had not been exposed to fibroblasts were treated in the same manner.

The effect of the various media on alveolar type II cells, that had attached during a 1-night adherence period [33] in MEM containing 10% fetal bovine serum, was determined by aspirating the old medium, rinsing the cells with serum-free MEM, and subsequently incubating them for 20 h in each of the media pre-mixed 1:1 with serum-free MEM. These 1:1 dilutions are hereafter referred to as control MEM (prepared with non-conditioned MEM), MEM/cort (with cortisol-containing MEM), MEM/fib (with fibroblast-conditioned MEM) and MEM/cort/fib (with fibroblast-conditioned, cortisol-containing MEM). The MEM used in these experiments contained 100 U/ml penicillin, 100 μg/ml kanamycin and 2.5 μg/ml amphotericin B.

2.3. *Fatty acid synthase assay*

After exposure to control MEM or MEM/cort/fib, cells on 10-cm dishes were rinsed 3 times with 10 mM Tris, 0.25 M sucrose (pH 7.4) and scraped from the dishes in a small volume of this buffer, using a rubber policeman. They were disintegrated by sonication (4 × 15 s at 0°C; frequency 23 kHz; amplitude 6 μm). Fatty acid synthase activity in the sonicates was measured spectrophotometrically [15]. The protein content of the sonicates was determined according to Lowry et al. [34], using bovine serum albumin as the standard.

2.4. *Isolation and Northern blot hybridization of RNA*

After exposure to various media, cells on 10-cm dishes were rinsed with phosphate-buffered saline (pH 7.4) and subsequently scraped from the dishes in 4 M guanidinium thiocyanate, 25 mM sodium citrate, 0.5% sodium *N*-laureoylsarcosine, 0.1% antifoam A, 0.1 M β-mercaptoethanol [35] (3 ml per 6 dishes). The viscous suspension was homogenized with an Ultra-Turrax mixer (Janke and Kunkel, Staufen i.Br., Germany) at 0°C. From this homogenate the RNA was separated by CsCl centrifugation [35]. RNA was quantified by measuring the absorbance at 260 nm. Aliquots containing 50 μg total RNA were resolved by electrophoresis through a 1.2% agarose–formaldehyde gel [36]. 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 [18] 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 [37] 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 [18] 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. FAS, malic enzyme, ACC, ACL and CPCT mRNAs were analyzed on fresh blots. After washing of the blots for 1 h at 100°C in 0.05 × SSPE, 1% sodium dodecyl sulfate to remove the probe, they were re-hybridized with β-actin probe and subsequently with glyceraldehydephosphate dehydrogenase probe.

3. RESULTS

3.1. *Fatty acid synthase activity*

In a previous study we observed that exposure of fetal rat type II cells to MEM/cort/fib instead of control MEM led to an enhancement of fatty acid synthesis by 50% [16]. In the present investigations we exposed the fetal type II cells to the same medium and subsequently measured the activity of FAS in sonicated cells. In the sonicated control cells (cells cultured for 20 h in control MEM) the specific activity of FAS was 1.47 ± 0.34 nmol/min·mg protein (mean ± S.E.M.; n=6). Because of the variability of this control value, the FAS activity upon MEM/cort/fib exposure was calculated as a percentage of the control value for each experiment. In the 6 experiments the activity in the type II cells exposed to MEM/cort/fib was 208 ± 37% (mean ± S.E.M.) of the activity in cells exposed to control MEM (P<0.005 according to Student's t-test). The previously observed [16] rise in the rate of fatty acid synthesis is therefore accompanied by a significantly enhanced FAS activity.

3.2. *Abundance of the mRNAs for FAS, ACC, ACL and malic enzyme*

As we had no way to measure the absolute quantities of the mRNAs, we measured only relative levels. For this purpose equal amounts of total RNA were analyzed by Northern blot hybridization. Table I shows that exposure of the type II cells to MEM/cort/fib instead of control MEM led to a significant rise in the abundance of FAS mRNA, indicating that the increased FAS activ-

ity observed under this condition was at least partially due to pre-translational regulation. Exposure to MEM/cort/fib instead of control MEM also led to increased abundance of the mRNAs encoding ACC and ACL (Table I). No change was seen in the abundance of malic enzyme mRNAs (Table I). This is reminiscent of the results obtained in a study on the developmental patterns of the mRNAs of lipogenic enzymes in fetal rat lung. In that study [18] pre-translational regulation of FAS, ACC and ACL, but not of malic enzyme, was observed.

It can be seen from Table I that the increase in the abundance of FAS mRNA due to exposure to MEM/cort/fib was not found upon exposure to either MEM/cort or MEM/fib. The abundance of ACC mRNA and ACL mRNA was significantly higher than the control value upon exposure to MEM/cort but, as in the case of FAS mRNA, the values seen after exposure to MEM/cort/fib were significantly higher than those seen after exposure to each of the other media. This indicates that both the cortisol in the medium and fibroblast conditioning are necessary for the maximal effect. The small increasing effect of MEM/cort exposure on ACC and ACL mRNA in the type II cell preparations may be due to contaminating fibroblasts.

That the effect on the abundance of FAS, ACC and ACL mRNA in the fetal type II cells is not an aspecific effect on mRNAs in general can be concluded from the absence of an effect on the mRNAs of malic enzyme, glyceraldehydephosphate dehydrogenase and β-actin (Table I). The latter two are often used as internal controls in studies like the present one.

3.3. *Changes in the abundance of CPCT mRNA*

Table I shows that exposure of fetal type II cells to MEM/cort/fib instead of control MEM does not only lead to an increased abundance of the mRNAs of FAS, ACC and ACL, but also to that of the 5 and 1.5 kb

Table I

Relative abundance of mRNAs in fetal type II cells after exposure to various culture media

mRNA	Relative abundance (% of value after exposure to control MEM (condition 1))		
	MEM/cort (condition 2)	MEM/fib (condition 3)	MEM/cort/fib (condition 4)
Fatty acid synthase	134.3±10.4 (5) [4]	135.6±21.3 (5) [4]	200.3±38.2 (7) [1,2,3]
Acetyl-CoA carboxylase	133.2±2.5 (5) [1,4]	123.2±7.5 (5) [4]	185.7±18.7 (7) [1,2,3]
ATP citrate lyase	125.7±4.0 (5) [1,3,4]	99.0±11.5 (5) [2,4]	160.8±11.6 (7) [1,2,3]
Malic enzyme			
3.4 kb	104.9±11.0 (5)	128.0±19.9 (5)	119.9±12.3 (6)
2.4 kb	111.9±8.1 (5)	116.3±17.3 (5)	92.3±8.9 (6)
Glyceraldehydephosphate dehydrogenase	107.7±3.1 (5)	108.8±4.1 (5)	109.8±4.1 (6)
β-Actin	100.6±2.4 (5)	107.2±2.8 (5)	94.8±3.2 (6)
CTP: phosphocholine cytidylyltransferase			
5 kb	108.1±6.5 (5) [4]	96.8±6.8 (5) [4]	128.3±9.7 (6) [1,2,3]
1.5 kb	121.9±6.3 (5) [n.s.]	113.5±5.3 (5) [4]	136.5±12.4 (6) [1,3]

RNA was isolated as described in section 2 from type II cells exposed to control MEM (condition 1), MEM/cort (condition 2), MEM/fib (condition 3) or MEM/cort/fib (condition 4) and analyzed by Northern blot hybridization. For the various proteins the mean value for the mRNA upon exposure of type II cells to control MEM had an S.E.M. of 4–17%. For each experiment the relative abundance of a particular mRNA was expressed as a percentage of the abundance after exposure to control MEM. The values in the table represent the mean ± S.E.M. of these latter percentages. The figures in parenthesis indicate the number of experiments. In 5 experiments all four conditions were included. In 2 other experiments only conditions 4 and 1 were compared. Each experiment was carried out with a separate cell preparation. Statistical analysis was carried out by analysis of variance. Only in those cases where the F-test indicated that there was a significant difference ($F<0.05$) among exposure conditions, were means compared pair-wise by Student's t-test. The numbers in square brackets indicate for each condition from which other conditions its value differs significantly according to the t-test ($P<0.05$). [n.s.], not significantly different ($P>0.05$) from any other condition according to the t-test. No significant difference among exposure conditions was indicated by the F-test for the mRNAs of malic enzyme, glyceraldehydephosphate dehydrogenase and β-actin.

mRNAs of CPCT. The abundance of the CPCT 5 kb mRNA upon exposure to MEM/cort/fib is significantly higher than that upon exposure to each of the other media, indicating the importance of both the cortisol and the fibroblast conditioning for maximal effect. MEM/cort exposure caused a (not statistically significant) increase in the abundance of CPCT 1.5 kb mRNA. As a result, for this 1.5 kb mRNA, the additional effect of fibroblast-conditioning was not statistically significant.

4. DISCUSSION

The present experiments show for the first time that exposure to fibroblast-conditioned, cortisol-containing medium causes an increased FAS activity in isolated fetal type II cells, and that this effect is at least partially brought about by pre-translational regulation. The same medium also increases the abundance of the mRNAs of two other enzymes involved in fatty acid synthesis, ACC and ACL. These data indicate that pre-translational regulation of FAS, ACC and ACL is probably involved in the increased rate of fatty acid synthesis observed in fetal type II cells upon exposure to fibroblast-conditioned, cortisol-containing medium [16] and in whole lung upon glucocorticoid exposure [21]. The observation that the cortisol effect on the abundance of FAS, ACC and ACL mRNAs is significantly less when the cortisol-containing medium is not conditioned by lung fibroblasts indicates that mesenchymal–epithelial interaction is involved in the induction of fatty acid synthesis. The FPF that has been described as stimulating DSPC synthesis [8–10] and CPCT activity [11] in fetal type II cells may also be involved in the stimulation of fatty acid synthesis in these cells. However, it should be noted that our experiments do not distinguish between (i) an effect via a factor produced in the fibroblasts upon exposure to cortisol, and (ii) an effect via a combination of cortisol and a factor that is secreted by the fibroblasts even if they are not exposed to cortisol.

The regulation of CPCT activity and its intracellular site of action are incompletely known. A number of mechanisms for its activation have been proposed, including translocation from cytosol to endoplasmic reticulum [38] and activation by fatty acids and phospholipids [24,25]. Studies with whole lung tissue on hormonal stimulation of PC synthesis have yielded a considerable body of evidence in favor of a regulatory role of the cytosolic part of the enzyme in pulmonary PC metabolism (see [39] for review). On the other hand, upon administration of dexamethasone to pregnant rats, Post [14] observed that the increased rate of PC synthesis in the type II cells isolated from the lungs of the fetuses was accompanied by an increased microsomal CPCT activity without a change in the cytosolic activity. Recently, Rooney et al. [23] observed that glucocorticoids increased the activity but not the amount of CPCT in explants of fetal rat lungs. Based on this and other observations (see section 1) they suggested that the stimulatory effect of glucocorticoids on CPCT activity in fetal lung is secondary to an acceleration of the synthesis of fatty acids which, either in free form or after incorporation into phospholipids, activate existing CPCT molecules. Our earlier observation [16] that fibroblast-conditioned, cortisol-containing medium stimulates fatty acid synthesis in type II cells is in agreement with this hypothesis. In view of the present observation that fibroblast–type II cell interaction is involved in the pre-translational regulation of FAS, ACC and ACL it is very well possible that the stimulation of CPCT activity in fetal type II cells by FPF [11] results, at least partially, from increased fatty acid synthesis brought about by this factor.

Although the observations on the pre-translational regulation of fatty acid synthesis are in agreement with the hypothesis that glucocorticoids increase the activity of CPCT via induction of fatty acid synthesis, we found that exposure of fetal type II cells to fibroblast-conditioned, cortisol-containing medium does not only lead to an increased rate of fatty acid synthesis in these cells, but also to increased abundance of CPCT mRNA (Table I). Though the latter effect is less pronounced, it does suggest that the increased CPCT activity seen in type II cells upon exposure to this medium [11], and in lung tissue upon glucocorticoid treatment [20,40], is not only due to stimulation by an increased amount of fatty acids or phospholipids synthesized therefrom, but also, in part, to pre-translational regulation of CPCT itself. It is of note that in the experiments by Rooney et al. [23], in which an effect of glucocorticoid on CPCT activity, but not on CPCT amount, in fetal lung explants was observed, only the cytosolic fraction was studied. The possibility that pre-translational regulation of CPCT is involved in the enhancement of the CPCT activity observed in the microsomal fraction upon dexamethasone exposure of fetal rat lung explants [40] cannot be excluded. Pre-translational regulation of CPCT was recently also observed in a macrophage cell-line [41].

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