Fatty acids stimulate phosphatidylcholine synthesis and CTP:choline-phosphate cytidylyltransferase in type II pneumocytes isolated from adult rat lung

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The regulation by cyclic AMP and fatty acids of phosphatidylcholine (PC) synthesis in rat alveolar type II cells was studied. In contrast with results with hepatocytes, cyclic AMP and its potent chlorophenylthio analogue had no inhibitory effect on [*Me*-¹⁴C]choline incorporation into PC in pulse-chase studies with alveolar type II cells. The inclusion of the fatty acids palmitate, oleate or linoleate in the chase incubation medium stimulated the incorporation of [*Me*-¹⁴C]choline into PC by type II cells. The effect of palmitate, which was more pronounced than that of the other fatty acids, appeared to be concentration-dependent. Increased [*Me*-¹⁴C]choline incorporation into PC was paralleled by an accelerated disappearance of the radiolabel from choline phosphate, which is consistent with an activation of CTP:choline-phosphate cytidylyltransferase. This enzyme is considered to be rate-limiting in the synthesis of PC *de novo* by type II cells. As fatty acids are also substrate for PC synthesis, their effect could also be due to compensation for a fatty acid deficiency. To test this possibility, fatty acid synthesis in the type II cells was stimulated by addition of lactate. Even then, an additional stimulation of PC synthesis by palmitate was observed, which supports the regulatory influence of exogenous fatty acids. Incubation of type II cells in the presence of 0.2 mM-palmitate resulted in a 45% increase in the membrane-bound CTP:choline-phosphate cytidylyltransferase activity, whereas the soluble activity remained unchanged. Choline kinase activity in the soluble fraction increased by 48%. However, the increase in choline kinase is unlikely to be responsible for the increased metabolic flux through the choline phosphate pathway, because there is a relatively large pool of choline phosphate in type II cells. Therefore it is suggested that the microsomal CTP:choline-phosphate cytidylyltransferase is the form of this enzyme which is active in surfactant PC synthesis, and possibly has a regulatory role in this pathway.

INTRODUCTION

Phosphatidylcholine (PC) is the predominant phospholipid class of pulmonary surfactant. Its most abundant species is dipalmitoyl-PC, which is of prominent importance in lowering the surface tension at the alveolar air/water interface to a value sufficiently low to guarantee alveolar stability during the respiratory cycle (Goerke, 1974). PC is synthesized together with the other components of surfactant in alveolar type II cells.

The major, if not exclusive, route of choline incorporation into surfactant PC is the Kennedy pathway (Rooney, 1985; Harwood, 1987; Van Golde *et al.*, 1988), proceeding via choline phosphate and CDP-choline. Pulse-chase studies with radiolabelled choline (Post *et al.*, 1982) and measurements of the pool sizes of the intermediates (Post *et al.*, 1984) have led to the conclusion that the conversion of choline phosphate into CDP-choline by CTP:choline-phosphate cytidylyltransferase (EC 2.7.7.15) (cytidylyltransferase) is the rate-limiting step governing the rate of PC synthesis by alveolar type

II cells. This is consistent with the results of investigations in other cell systems (for a review see Pelech & Vance, 1984). Cytidylyltransferase occurs in both the cytosolic and the microsomal fractions. The cytosolic form of the enzyme in liver (Fiscus & Schneider, 1966) and lung (Stern *et al.*, 1976) is susceptible to activation by various lipids. Activation by phospholipid vesicles was correlated with the ability of the vesicles to bind cytidylyltransferase (Feldman *et al.*, 1985). It has been suggested that the cytosolic enzyme is an inactive storage form which can be activated by its translocation and non-covalent association with the membranes of the endoplasmic reticulum (Vance & Pelech, 1984). Purified cytidylyltransferase from rat liver cytosol has been shown to have hydrophobic properties similar to those of membrane proteins (Weinhold *et al.*, 1986). In hepatocytes and HeLa cells the subcellular distribution of cytidylyltransferase, and thereby its activation state, appear to be controlled by the concentration of fatty acids and by cyclic-AMP-dependent protein phosphorylation (Pelech *et al.*, 1983; Vance & Pelech, 1984).

Abbreviations used: PC, phosphatidylcholine; BSA, bovine serum albumin; cytidylyltransferase, CTP:choline-phosphate cytidylyltransferase (EC 2.7.7.15).

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Evidence for a similar regulatory mechanism of cytidylyltransferase has been found in studies on fetal lung. At 3 h after premature delivery of rat fetuses at 20 and 21 days of gestation, there was an increase in the incorporation of radiolabelled choline into PC by slices of the fetal lungs, which was correlated with increased activity of microsomal cytidylyltransferase (Weinhold *et al.*, 1981). In a subsequent study the increased microsomal cytidylyltransferase activity in prematurely delivered 21-day fetuses was also correlated with an increased fatty acid content of the lung microsomes (Weinhold *et al.*, 1984). A study on fetal rabbit lung cytidylyltransferase *in vitro* demonstrated inhibition of the enzyme after incubation with MgATP, suggesting a possible regulation by cyclic-AMP-dependent protein kinase (Radika & Possmayer, 1985). In experiments with isolated fetal rabbit type II pneumocytes, exposure to fatty acids (palmitate and oleate) was shown to enhance choline incorporation into PC and cytidylyltransferase activity in the microsomal fraction (Aeberhard *et al.*, 1986). Post (1987) demonstrated increased microsomal-associated activity of cytidylyltransferase in organotypic cultures of lung cells from 19-day-fetal rats after incubation of the cultures with cortisol, and in isolated fetal type II cells after maternal dexamethasone treatment. Contradictory findings have been reported by Rooney *et al.* (1986), who found that dexamethasone stimulates the cytidylyltransferase activity predominantly in the supernatant fraction of cultured fetal rat lung explants. In a study with choline-depleted adult rat type II pneumocytes, Tesan *et al.* (1985) demonstrated an increase of cytidylyltransferase activity which was due to increased activity in the particulate fraction. However, neither in choline-depleted cells nor in choline-repleted cells were cyclic AMP analogues or fatty acids found to have an effect on cytidylyltransferase activity.

We attempted to gain further insight into the physiological regulation of PC synthesis and cytidylyltransferase activity in adult rat lung type II cells by studying these cells under conditions similar to those used in studies with hepatocytes (Pelech *et al.*, 1981, 1983).

METHODS AND MATERIALS

Cell isolation

Type II cells were isolated from adult male Wistar rats (180–220 g). For pulse-chase studies on the incorporation of [*Me*-¹⁴C]choline by type II cells in primary culture we used the method described by Mason *et al.* (1977), involving trypsin treatment of the lungs followed by discontinuous albumin-density-gradient centrifugation and differential adherence in primary culture. Cell yield, purity and viability were in the same range as reported previously (Batenburg *et al.*, 1978).

For other experiments we employed type II cells freshly isolated by the procedure described by Dobbs *et al.* (1986). In this method lung tissue is digested with elastase and the resulting cell suspension is panned on bacteriological-grade Petri dishes coated with rat IgG. With this method the average cell yield was $(19 \pm 2) \times 10^6$ per rat, the percentage of type II cells in the final cell suspension was $90 \pm 3\%$ and the viability (estimated by Trypan Blue exclusion) $90 \pm 4\%$.

Rat hepatocytes were isolated by the method of Seglen (1976), as modified by Geelen *et al.* (1978), and cultured

in arginine-free Dulbecco's modified Eagle's medium with 0.4 mM-ornithine, 100 nM-insulin, 100 µg of streptomycin/ml, 100 units of penicillin G/ml, 10 mM-Hepes and 20% (v/v) fetal-calf serum (Pritchard & Vance, 1981).

Incubation of isolated cells

Pulse-chase studies of the effect of chlorophenylthio cyclic AMP on the incorporation of [*Me*-¹⁴C]choline into PC by isolated type II cells and hepatocytes in primary culture were carried out as described by Pelech *et al.* (1981) in arginine-free Dulbecco's modified Eagle's medium with 0.4 mM-ornithine. The cells were preincubated for 1 h to adapt to the medium. For the 1 h pulse incubation the unlabelled choline in the medium was replaced by 28 µM-[*Me*-¹⁴C]choline (sp. radioactivity 35 500 d.p.m./nmol for type II cells; 17 700 d.p.m./nmol for hepatocytes). For the subsequent chase incubation, the radioactive tracer was again replaced by an equal concentration of unlabelled choline and 0.5 mM-chlorophenylthio cyclic AMP, 0.2 mM-isobutylmethylxanthine, or both, were added. The incubations were performed in air/CO₂ (19:1).

Pulse-chase experiments on the effects of fatty acids on the metabolism of [*Me*-¹⁴C]choline in type II cells in primary culture were performed as described by Post *et al.* (1982), with a 1 h pulse incubation with 25 µM-[*Me*-¹⁴C]choline (59 200 d.p.m./nmol). Fatty acids were complexed to bovine serum albumin (BSA) in a molar ratio of 5.3:1 and added to the chase incubation medium. Incubations were stopped by scraping the cells into methanol/water (5:4, v/v).

Experiments on the effects of lactate and palmitate on [*Me*-¹⁴C]choline incorporation into PC by freshly isolated type II cells were performed in Krebs-Ringer bicarbonate buffer (pH 7.4) with 5 mM-glucose as described by Maniscalco *et al.* (1983). Incubation tubes were flushed with air/CO₂ (19:1) every 15 min. [*Me*-¹⁴C]Choline (25 µM; 88 000 d.p.m./nmol) was present during the entire incubation period, which was terminated by placing the tubes on ice and centrifuging them. The cells were washed twice with unlabelled buffer. Lipids were extracted as described by Bligh & Dyer (1959), and the radioactivity incorporated into PC and choline phosphate was determined after t.l.c. as described by Post *et al.* (1982).

Cell fractionation

Experiments on the effect of palmitate on enzyme activities were performed with freshly isolated type II cells. These were incubated for 1 h in Krebs-Ringer bicarbonate buffer (pH 7.4) equilibrated with air/CO₂ (19:1). The medium included either 0.2 mM-palmitate (bound to BSA in a molar ratio of 5.3:1) or a matched concentration (0.256%, w/v) of fatty acid-free BSA (controls). The incubations were terminated by placing the tubes on ice. After removal of the medium by centrifugation, the cells were homogenized in 150 mM-NaCl/5 mM-Tris/HCl, pH 7.4, for determination of choline kinase, or in 150 mM-NaCl/6.6 mM-EDTA/20 mM-Tris/HCl, pH 7.5, for determination of cytidylyltransferase. For homogenization, a Dounce glass/glass homogenizer was used (10 strokes with the loose-fitting pestle, 50 strokes with the tight-fitting pestle). In initial experiments the homogenate was centrifuged for 5 min at 130 000 g in a Beckmann Airfuge. The supernatant

was designated 'soluble fraction'. The pellet was resuspended in the homogenization buffer and designated 'membrane fraction'. However, this procedure resulted in a high contamination of the membrane fraction by cytosolic protein. As this contamination was probably due to unbroken cells, the method was refined by first centrifuging the homogenate at 14 550 *g* for 5 min and discarding the pellet, presumed to contain unbroken cells, mitochondria, nuclei and debris. The 14 550 *g* supernatant was transferred to the Airfuge and processed as described above.

Enzyme activity and protein determinations

CTP:choline-phosphate cytidylyltransferase activity was determined by measurement of the conversion of [*Me*-¹⁴C]choline phosphate into [¹⁴C]CDP-choline under the incubation conditions described by Weinhold *et al.* (1986). In the soluble fractions the enzyme was measured in the absence and in the presence of 1 mM-phosphatidylglycerol as activator. After 10 min incubation at 37 °C the reaction was stopped by boiling. CDP-choline and choline phosphate were separated by t.l.c., and the radioactivity in CDP-choline was determined as described by Post *et al.* (1982). Under the conditions used, the formation of [¹⁴C]CDP-choline from [*Me*-¹⁴C]choline phosphate by subcellular fractions from isolated type II cells was constant with time and proportional to the amount of protein.

Choline kinase (EC 2.7.1.32) activity was assayed by a modification of the method of Ulane *et al.* (1977). The formation of [¹⁴C]choline phosphate from [*Me*-¹⁴C]choline was determined. The assay buffer contained 50 mM-NaCl, 50 mM-ATP, 67 mM-Tris/HCl (pH 8.5), 100 mM-MgCl₂, 5 mM-[*Me*-¹⁴C]choline and 6–27 µg of cytosolic protein in a final volume of 60 µl. After 10 min incubation at 37 °C the reaction was stopped by boiling. Choline and choline phosphate were separated by paper chromatography. Radioactivity in choline phosphate was determined by liquid-scintillation counting. Under the conditions used the formation of choline phosphate was linear with time and proportional to the amount of protein.

Protein was measured with the Bio-Rad (Munich, Germany) reagent as described by Bradford (1976).

Materials

Rats were obtained from the Central Institute for the Breeding of Laboratory Animals, Zeist, The Netherlands. [*Me*-¹⁴C]Choline phosphate (sp. radioactivity 55 Ci/mol) was obtained from NEN Chemicals (Dreieich, Germany) and [*Me*-¹⁴C]choline (sp. radioactivity 54 Ci/mol) from Amersham International (Amersham, Bucks., U.K.). Phosphatidylglycerol (derived from egg phosphatidylcholine) was bought from Sigma (St. Louis, MO, U.S.A.).

RESULTS

Effect of chlorophenylthio cyclic AMP on PC synthesis

Fig. 1 shows the results of a pulse-chase experiment on the incorporation of [*Me*-¹⁴C]choline into PC in which isolated hepatocytes and alveolar type II cells in primary culture were incubated under identical conditions. The addition of 0.5 mM-chlorophenylthio cyclic AMP to the chase medium markedly decreased the incorporation of [*Me*-¹⁴C]choline into PC by hepatocytes, as has been shown previously by Pelech *et al.* (1981). In type II cells

Fig. 1. Effect of chlorophenylthio cyclic AMP on the incorporation of [*Me*-¹⁴C]choline into PC by hepatocytes and alveolar type II cells

Pulse-chase incubations of hepatocytes (a) and alveolar type II cells (b) in primary culture were performed in arginine-free Dulbecco's modified Eagle's medium with 0.4 mM-ornithine as described in the Methods and materials section. ○, Chlorophenylthio cyclic AMP (0.5 mM) present in the chase medium; ●, controls. The data are representative of results obtained with two (hepatocytes) and five (type II cells) preparations respectively.

in primary culture, on the other hand, chlorophenylthio cyclic AMP did not inhibit PC synthesis (Fig. 1). In a series of five experiments there was a tendency for a slight stimulation (mean 9% over 1.5 h and 6% over 3 h) of PC synthesis. In two of the experiments, the addition of chlorophenylthio cyclic AMP in combination with 0.2 mM-isobutylmethylxanthine (a phosphodiesterase inhibitor) was tested and did not exert an inhibitory effect on [*Me*-¹⁴C]choline incorporation into PC by the type II cells (results not shown). Also, other experimental protocols were used to ascertain the absence of an inhibitory effect of cyclic AMP or its potent analogue on PC synthesis in type II cells. In simple label-incorporation studies and in pulse-chase experiments with a pulse period of 15 min, neither cyclic AMP (0.5 mM) nor chlorophenylthio cyclic AMP (0.5 mM) nor the combination of either with 0.2 mM-isobutylmethylxanthine

Fig. 2. Effects of fatty acids on the incorporation of [$Me-^{14}C$]-choline into PC and choline phosphate by type II cells

Pulse-chase incubations of type II cells in primary culture were performed as described in the Methods and materials section: (a) and (c), label in PC; (b) and (d), label in choline phosphate. In (a) and (b), palmitate (bound to BSA in a constant molar ratio of 5.3:1) was added to the chase incubation medium in the following concentrations: 0.002 mM ($\square$), 0.01 mM ($\blacktriangle$), 0.05 mM ($\circ$), 0.2 mM ($\blacksquare$) and 1.0 mM ($\triangle$); $\bullet$, controls without palmitate in the chase medium. In (c) and (d) the following fatty acids (bound to BSA in a molar ratio of 5.3:1) were added to the chase medium in a concentration of 0.2 mM: $\circ$, palmitate; $\square$, oleate; $\triangle$, linoleate; $\bullet$, controls without fatty acids in the chase medium.

exerted an inhibitory effect on [$Me-^{14}C$]choline incorporation into PC by type II cells in primary culture (results not shown).

Effects of fatty acids on PC synthesis

The effect of palmitate on the incorporation of [$Me-^{14}C$]choline into PC by type II cells in primary culture was studied in a pulse-chase experiment. Different concentrations of palmitate were included in the chase medium. Fig. 2(a) shows the resulting dose-dependent stimulation of PC synthesis. The accelerated appearance of label in PC was paralleled by an accelerated disappearance of label from choline phosphate (Fig. 2b). In a similar pulse-chase experiment the effect of palmitate was compared with that of oleate and linoleate in equal concentrations (0.2 mM). Figs. 2(c) and 2(d) show that the effects of oleate and linoleate on PC synthesis and on the disappearance of radiolabel from choline phosphate are similar to those of palmitate, but much less pronounced. That the effect of oleate and linoleate is smaller than that of palmitate is not due to a faster utilization of the former fatty acids in esterification or oxidation reactions, as it has been shown that in type II cells oleate and linoleate are esterified more slowly than palmitate, and of the two unsaturated fatty acids only oleate is oxidized faster than palmitate (Stantcheva *et al.*, 1982). The stimulatory effect of palmitate on the

Fig. 3. Effects of lactate and palmitate on the incorporation of [$Me-^{14}C$]choline into PC by freshly isolated type II cells

The incubation conditions are given in the Methods and materials section. The medium was supplemented with 10 mM-lactate and 0.256% fatty acid-free BSA ($\blacksquare$), 0.2 mM-palmitate (bound to BSA in a molar ratio of 5.3:1) ($\circ$), 10 mM-lactate and 0.2 mM-palmitate (bound to BSA) ($\square$) or 0.256% fatty acid-free BSA only ($\bullet$). The data represent mean values $\pm$ S.E.M. obtained in three experiments with three different cell preparations. They have been analysed by regression analysis using sequential F-tests (Steel & Torrie, 1981). Pair-wise comparisons of treatments were done by Bonferroni *t* tests. These analyses showed that inclusion in the medium of palmitate, lactate or palmitate plus lactate significantly ($P < 0.001$) stimulated the incorporation of [$Me-^{14}C$]choline into PC, and that in the presence of lactate additional inclusion of palmitate also had a significant ($P < 0.002$) stimulatory effect.

conversion of [$Me-^{14}C$]choline into PC was also confirmed for freshly isolated type II cells in simple label-incorporation experiments (Fig. 3).

Effects of lactate on PC synthesis

In order to assess whether enhanced endogenous synthesis of fatty acids by type II cells has an effect on PC synthesis similar to that of increased exogenous supply of fatty acids, freshly isolated type II cells were incubated in Krebs-Ringer bicarbonate buffer containing 10 mM-lactate. Fig. 3 shows that the inclusion of lactate in the medium resulted in enhanced PC synthesis. The effects of 10 mM-lactate and 0.2 mM-palmitate on [$Me-^{14}C$]choline incorporation into PC in this cell preparation appeared to be additive.

Effects of palmitate on enzymes of the choline phosphate pathway

After incubation of freshly isolated type II cells for 1 h in the presence of 0.2 mM-palmitate, subcellular fractionation was performed and enzymes were measured in the subcellular fractions. Controls were incubated in

Table 1. Effect of palmitate on the activity of choline kinase and CTP:choline-phosphate cytidylyltransferase in subcellular fractions of type II cells

Freshly isolated type II cells were incubated for 1 h in the presence of 0.256% fatty acid-free BSA (control) or 0.2 mM-palmitate bound to BSA (molar ratio 5.3:1) before they were fractionated. +PG indicates that 1 mM-phosphatidylglycerol was present in the assay mixture. The data represent the mean values $\pm$ S.E.M. obtained in six experiments for each of the two enzymes, carried out with twelve different cell preparations. * and † indicate significant difference from control ($0.025 < P < 0.05$ and $0.01 < P < 0.025$ respectively) according to Student's *t* test for paired data.

Enzyme	Specific activity (nmol/min per mg of protein)		Ratio $\frac{+ \text{palmitate}}{\text{control}}$
	Control	+ Palmitate	
Choline kinase			
Soluble fraction	2.40 $\pm$ 0.47	3.55 $\pm$ 0.48*	1.48
Cytidylyltransferase			
Membrane fraction	2.47 $\pm$ 0.37	3.59 $\pm$ 0.51†	1.45
Soluble fraction	0.40 $\pm$ 0.07	0.43 $\pm$ 0.09	1.08
Soluble fraction (+PG)	1.25 $\pm$ 0.22	1.39 $\pm$ 0.22	1.11

the absence of fatty acids. Table 1 shows that palmitate caused a significant increase (by 48%) of the choline kinase activity in the soluble fraction.

We also measured the activity of cytidylyltransferase. Table 1 shows a marked increase (by 45%) in the membrane-bound component of the cytidylyltransferase activity as a result of incubation of the intact cells with palmitate, whereas incubation with palmitate did not result in a significant change in the soluble activity of this enzyme, regardless of whether this activity was measured in the presence or in the absence of the stimulator phosphatidylglycerol.

DISCUSSION

The results of the pulse-chase studies on the effect of chlorophenylthio cyclic AMP on choline incorporation into PC (Fig. 1) show that this potent cyclic AMP analogue has no inhibitory effect on PC synthesis in alveolar type II cells. This contrasts with the marked inhibition of PC synthesis found in hepatocytes, which is in agreement with the previous report by Pelech *et al.* (1981). As β -adrenergic agents are known to stimulate PC secretion by type II cells, an effect which is mediated by cyclic AMP (Brown & Longmore, 1981), a simultaneous inhibitory effect on PC synthesis would lead to a rapid depletion of intracellular surfactant stores. Therefore differences in the regulatory mechanisms of PC synthesis in the two cell systems have to be expected.

On the other hand, a similarity in the regulation of PC synthesis is suggested by the effect of fatty acids. As has been reported for hepatocytes (Pelech *et al.*, 1983), the presence of fatty acids also had a stimulatory effect on PC synthesis in type II cells. In these the stimulation was most pronounced for palmitate (Fig. 2). However, in hepatocytes it was greater with oleate (Pelech *et al.*, 1983). In bovine lymphocytes an enormous stimulation of PC synthesis was observed with unsaturated fatty acids (7-fold stimulation by 6.5 μ M-oleate), whereas palmitate caused an increase of 66% and stearate and arachidate had no effect (Anderson *et al.*, 1985). Finally, in the isolated perfused hamster heart stearate enhanced

PC synthesis, but oleate and arachidonate had no effect (Mock *et al.*, 1986). Thus there appears to considerable variation in the susceptibility of specific cell types to stimulation of PC synthesis by specific fatty acids, which cannot be explained at present. Alveolar type II cells are able to synthesize fatty acids from various precursors. Batenburg *et al.* (1978) have shown that, in the absence of fatty acids from the incubation medium, glucose-derived carbon atoms are effectively incorporated into the fatty-acyl moieties of PC. Nevertheless, under suboptimal culture conditions a fatty acid deficiency might develop in these cells and might limit the supply of diacylglycerols for the final cholinephosphotransferase (EC 2.7.8.2) step in PC synthesis. To study this possibility, we compared the PC synthesis in rat type II cells under control conditions with that under conditions in which the endogenous fatty acid synthesis was stimulated by addition of lactate. The latter conditions were identical with those shown by Maniscalco *et al.* (1983) to be optimal for fatty acid synthesis in freshly isolated rabbit type II cells. Even in the presence of lactate the addition of palmitate resulted in a stimulation of PC synthesis. This suggests that at least part of the effect of palmitate on PC synthesis is due to a regulatory influence on the choline phosphate pathway. However, the results have to be interpreted with caution, because it is not known whether endogenous fatty acid synthesis, even at an optimal rate, is sufficient for an optimal supply of diacylglycerols for PC synthesis.

The accelerated decrease in the labelling of choline phosphate which was shown when fatty acids were included in the chase incubation medium in pulse-chase studies (Figs. 2b and 2d) is consistent with regulation of the choline phosphate pathway at one of the steps converting choline phosphate into PC. As the choline phosphotransferase reaction is near equilibrium (Infante, 1977), the more likely regulatory enzyme is cytidylyltransferase. Enzyme measurements were performed in subcellular fractions of type II cells after incubation of the cells for 1 h with or without palmitate. There was a considerable increase in the membrane-bound activity of cytidylyltransferase, which paralleled the stimulation of

PC synthesis. The soluble cytidylyltransferase activity did not change (Table 1). There was also a significant increase in choline kinase in the soluble fraction (Table 1). However, it is unlikely that this increase had any influence on the metabolic flux through the choline phosphate pathway, because type II cells are known to contain a relatively large pool of choline phosphate under the experimental conditions used (Post *et al.*, 1984). Thus the results are consistent with the hypothesis that the flux through the choline phosphate pathway in type II cells is regulated by the activity of microsomal cytidylyltransferase. Similarly, increases in microsomal (or membrane-bound) cytidylyltransferase activity have been found to accompany the stimulation of PC synthesis by fatty acids in rat hepatocytes (Pelech *et al.*, 1983), bovine lymphocytes (Whitlon *et al.*, 1985) and hamster heart (Mock *et al.*, 1986). In our study a parallel decrease in the cytosolic activity of cytidylyltransferase could not be demonstrated. A possible decrease in cytosolic activity may have been too small to be detected with the methods employed. Thus the validity of the translocation hypothesis (Vance & Pelech, 1984) for alveolar type II cells remains conceivable. However, allosteric activation of the microsomal enzyme by a change in its membrane environment induced by fatty acids is an alternative possibility.

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