Fatty acids reverse the cyclic AMP inhibition of triacylglycerol and phosphatidylcholine synthesis in rat hepatocytes

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The influence of cyclic AMP analogues and fatty acids on glycerolipid biosynthesis in monolayer cultures of rat hepatocytes was investigated. Chlorophenylthio-cyclic AMP and adenosine 3':5'-cyclic phosphorothioate inhibited the rate of triacylglycerol synthesis from [1(3)-³H]glycerol, and phosphatidylcholine synthesis from [*Me*-³H]-choline. Supplementation of the hepatocytes with palmitate (1 mM) reversed chlorophenylthio-cyclic AMP inhibition of triacylglycerol synthesis. Similarly, cyclic AMP analogue-inhibition of phosphatidylcholine synthesis was abolished when the cells were simultaneously incubated with oleate (3 mM). Reactivation of phosphatidylcholine synthesis in chlorophenylthio-cyclic AMP-supplemented cells with oleate was accompanied by conversion of CTP:phosphocholine cytidyltransferase into the membrane-bound form, since these cells released the enzyme more slowly after treatment with digitonin. The opposing actions of cyclic AMP and fatty acids are discussed in relation to the regulation of glycerolipid biosynthesis during starvation, diabetes and stress.

The importance of reversible protein phosphorylation for regulation of hepatic glycerolipid synthesis has been established only recently (Geelen *et al.*, 1980). There is preliminary evidence for inhibition of rat liver glycerol 3-phosphate acyltransferase by phosphorylation (Nimmo, 1980). Evidence for phosphorylation *in vitro* of phosphatidate phosphohydrolase (Berglund *et al.*, 1982), diacylglycerol acyltransferase (Haagsman *et al.*, 1981, 1982), phosphatidylethanolamine methyltransferase (Castano *et al.*, 1980) and phosphocholine cytidyltransferase (Pelech & Vance, 1982) has also been obtained. However, there is as yet no proof that these enzymes are directly phosphorylated by protein kinases *in vivo*. Still, the activities of glycerol 3-phosphate acyltransferase (Soler-Argilaga *et al.*, 1977; Nimmo, 1980), diacylglycerol acyltransferase (Haagsman *et al.*, 1981) and cytidyltransferase (Pelech *et al.*, 1981) in perfused rat liver or isolated hepatocytes are reduced

in response to glucagon or cyclic AMP analogue treatment, whereas phosphatidylethanolamine methyltransferase activity is stimulated (Castano *et al.*, 1980; Pritchard *et al.*, 1981).

Geelen *et al.* (1978) have examined the influence of glucagon on the incorporation of various radio-labelled precursors into the triacylglycerol, phosphatidylcholine and phosphatidylethanolamine of isolated rat hepatocytes. Glucagon did not affect [2-¹⁴C]glycerol incorporation into triacylglycerol and actually stimulated its appearance into phospholipid. [1-¹⁴C]Palmitate incorporation into triacylglycerol was reduced 37%, whereas entry into phosphatidylcholine and phosphatidylethanolamine was unchanged by glucagon. In a subsequent study, Geelen *et al.* (1979) reported that glucagon reduced [³²P]phosphate incorporation into phosphatidylcholine, but did not significantly inhibit [*Me*-¹⁴C]-choline appearance into phosphatidylcholine. We have since demonstrated that treatment of isolated rat hepatocytes with cyclic AMP analogues or aminophylline can be shown to restrict phosphatidylcholine synthesis from [*Me*-³H]choline when precautions are adopted to avoid isotope-dilution effects (Pelech *et al.*, 1981).

Since glycerol 3-phosphate acyltransferase, phos-

Abbreviations used: CPT-cAMP, 8-(4-chlorophenylthio)adenosine 3':5'-cyclic monophosphate; cAMPs, the *sp* isomer of adenosine 3':5'-cyclic phosphorothioate; Hepes, 4-(2-hydroxyethyl)-1-piperazine-ethanesulphonate.

phatidate phosphohydrolase and diacylglycerol acyltransferase are purportedly inhibited by phosphorylation, it is somewhat surprising that a marked inhibition of triacylglycerol synthesis is not evident after exposure of hepatocytes to glucagon. It may be that the supply of precursors is more critical for determining this rate of synthesis. Evidence for this supposition has been advanced by Declercq *et al.* (1982). These investigators reported that glucagon inhibited triacylglycerol synthesis in isolated hepatocytes by lowering their glycerol 3-phosphate content. Supplementation of the glucagon-treated cells with glycerol restored the rate of triacylglycerol formation.

In the starved rat, hormone-sensitive lipase catalyses the hydrolysis of adipose triacylglycerol, and the released fatty acids are delivered to the liver in a complexed form with albumin. Most of these fatty acids are ultimately oxidized to ketone bodies and CO₂ in the mitochondria. This arises because the glucagon and fatty acyl-CoA inhibition of acetyl-CoA carboxylase reduces the concentration of malonyl-CoA, which is a potent inhibitor of carnitine palmitoyltransferase I, the enzyme that facilitates fatty acid entry into the mitochondrial matrix (McGarry & Foster, 1979, 1980). Despite this, an increased amount of fatty acid also becomes available for hepatic glycerolipid synthesis during starvation. Tubbs & Garland (1964) have measured a doubling of the fatty acyl-CoA levels in the livers of 48 h-starved rats. Furthermore, the hepatic concentration of glycerol 3-phosphate can also increase with starvation and diabetes (McGarry & Foster, 1980; Zammit, 1981). It is also known that the synthesis and accumulation of triacylglycerol can increase in a number of conditions of metabolic stress including starvation and diabetes (Brindley, 1981; Brindley & Sturton, 1982). These conditions are characterized by low concentrations of circulating insulin relative to glucagon, catecholamines and glucocorticoids. These changes also result in increased synthesis of phosphatidate phosphohydrolase, which in the liver is induced by glucocorticoids and prevented by insulin (Lawson *et al.*, 1982).

Thus on the one hand glucagon may inhibit glycerolipid synthesis and direct fatty acids towards β -oxidation. However, the rate of triacylglycerol synthesis in the liver can increase in many situations where glucagon and cyclic AMP should be influential in controlling metabolism. These observations can be reconciled if high fatty acid concentrations are able to reverse the effects of glucagon and cyclic AMP on glycerolipid synthesis. We have found that fatty acids can indeed prevent the inhibition of triacylglycerol and phosphatidylcholine synthesis that is produced in hepatocytes by cyclic AMP analogues.

Experimental procedures

Materials and cells

cAMPS was a gift from Dr. F. Eckstein of the Max-Planck Institute for Experimental Medicine. CPT-cAMP was purchased from Boehringer-Mannheim. Oleate, palmitate and digitonin were bought from Sigma. Fatty acid-deficient bovine serum albumin was from Calbiochem. [*Me*-³H]-Choline, [1(3)-³H]glycerol and [1-¹⁴C]palmitate were obtained from Amersham International. Hepatocytes were isolated from female Wistar rats (125 g) by a collagenase-perfusion technique (Davis *et al.*, 1979) and resuspended in medium (1 × 10⁶/ml) as previously described (Pritchard & Vance, 1981). These cells were dispensed into collagen-coated plastic culture dishes (Lux Contur; 60 mm) (3 ml/dish) and incubated at 37°C under an atmosphere of air/CO₂ (19:1).

Incubation of hepatocytes with radiolabelled substrates

At 24 h after plating, hepatocytes were incubated for 1 h in serum-free medium (arginine-free Dulbecco's modified Eagle's medium with 28 μ M-choline chloride, 100 nM-insulin, 0.4 mM-ornithine, 100 μ g of streptomycin sulphate/ml, 100 units of penicillin G/ml, 10 mg of fatty acid-poor bovine serum albumin/ml and 10 mM-Hepes, pH 7.4). Cells were pulsed for 15–60 min before harvesting in the presence of 10 μ Ci of 200 μ M-[1(3)-³H]glycerol or 1.5 μ Ci of 200 μ M-[1-¹⁴C]palmitate with 0.5 mM-cyclic AMP analogue dissolved in serum-free medium. The concentration of glycerol was varied between 0 and 400 μ M in the ¹⁴C-labelled medium, whereas the concentration of palmitate ranged from 0 to 2 mM in the ³H-labelled medium. In other experiments, cells were pulsed for 30 min with 12.5 or 15 μ Ci of [*Me*-³H]choline (0.18 Ci/mmol) dissolved in serum-free medium. These pre-labelled cells were subsequently incubated for up to 2 h in the presence of 0.5 mM-cyclic AMP analogue and 0–4 mM-oleate in unlabelled medium. All the cells were scraped into 3.6 ml of ice-cold methanol/water (2:1, v/v) at the time of harvest, and the lipids were extracted upon addition of 4 ml of chloroform. A portion of the lower phase was applied to the origin of a Silica gel G-60 plate (20 cm × 20 cm; Merck) and the plate was developed in chloroform/methanol/acetone/acetic acid/water (10:2:4:2:1, by vol.) until the solvent front migrated 9 cm from the origin. The plate was dried and t.l.c. in the same direction was resumed in hexane/diethyl ether/acetic acid (60:40:1, by vol.) until the plate was fully developed. The lipids were visually detected with I₂ and the bands corresponding to diacylglycerol, triacylglycerol, phosphatidylcholine and phosphatidylethanolamine were separately scraped into

scintillation vials. Water (1 ml) and 10 ml of ACS fluid (Amersham) were also added, and the scintillation vials were shaken for at least 3 days before measuring radioactivity with an Isocap 300 liquid scintillation counter. The aqueous choline metabolites were resolved by t.l.c. on silica gel G60 with methanol/0.6% NaCl/NH₃ (sp.gr. 0.880) (10:10:1, by vol.) as the developing solvent.

The diacylglycerol levels in the isolated rat hepatocytes were determined by the method of Schneider (1977).

Digitonin-mediated release of cytidyltransferase from cultured rat hepatocytes

Monolayers of rat hepatocytes were rendered leaky essentially by the procedure of MacKall *et al.* (1979). Cells (2×10^6) in 60 mm dishes were washed once with 3 ml of ice-cold 0.145 M-NaCl after removal of the culture medium. The cells were incubated for up to 4 min with 1.5 ml of ice-cold digitonin (0.5 mg/ml) on a level surface with infrequent gentle agitation. The digitonin medium was carefully removed with a Pasteur pipette, the undissolved digitonin pelleted at 2500 g for 10 min, and the supernatant assayed for cytidyltransferase activity in the presence of 150 µg of total rat liver phospholipid and 100 µM-oleate (Pelech *et al.*, 1981).

Results and discussion

Influence of cyclic AMP analogues on glycerolipid synthesis

We were initially interested in the effect of cyclic AMP on glycerolipid synthesis in cultured mono-

layers of rat hepatocytes. We selected two analogues that are superior to dibutyryl-cyclic AMP, which requires metabolic transformation for activity (Drummond & Powell, 1970). CPT-cAMP has been shown to activate cyclic AMP-dependent protein kinase 100 times more effectively than cyclic AMP, and is 20-fold more stable to degradation by cyclic AMP phosphodiesterase (Miller *et al.*, 1975). The other analogue, cAMPS, differs from cyclic AMP by only a single atom; a sulphur atom replaces an oxygen in the phosphate ring (Eckstein, 1978).

When rat hepatocytes were exposed to CPT-cAMP or cAMPS in the pulse media, only [³H]glycerol incorporation into triacylglycerol was reduced ($P < 0.05$ after 60 min) (Fig. 1a). This inhibition of triacylglycerol synthesis was not evident when [¹⁴C]palmitate was used as the labelled precursor (results not shown). The pool size of diacylglycerol in control and CPT-cAMP-treated hepatocytes was measured so that the specific radioactivity of diacylglycerol could be determined. If the specific radioactivity of diacylglycerol was unchanged or increased, then the apparent inhibition of triacylglycerol synthesis could not be attributed to isotope-dilution effects. Since the pool size of diacylglycerol was not significantly affected after 1 h exposure of the cells to CPT-cAMP (Table 1), and the amount of [³H]glycerol label in the diacylglycerol pool was similar in untreated cells (Fig. 1b), the specific radioactivity of diacylglycerol was approximately the same in control and CPT-cAMP-treated cells. Consequently, CPT-cAMP and cAMPS may have inhibited the diacylglycerol acyltransferase-catalysed reaction and the actual

Fig. 1. The effects of cyclic AMP analogues on [¹⁽³⁾⁻³H]glycerol incorporation into hepatocyte glycerolipids. Monolayer cultures of rat hepatocytes were pre-incubated for 1 h in serum-free medium containing 100 nM-insulin and pulsed for 15–60 min with 10 µCi of 200 µM-[¹⁽³⁾⁻³H]glycerol in the absence (O) or in the presence of 0.5 mM-cAMPS (□) or 0.5 mM-CPT-cAMP (Δ). The cells were harvested as described in the Experimental procedures section and the radioactivity was determined in triacylglycerol (a) and in diacylglycerol (b). Each point represents the mean of three dishes, and the standard errors are indicated by bars (a) or were within 5% of the mean (b). This experiment was performed three times with similar results.

rate of triacylglycerol synthesis. Alternatively, the cyclic AMP analogues could have stimulated the catabolism of triacylglycerol (Debeer *et al.*, 1982).

CPT-cAMP and cAMPS may have also restricted the formation of diacylglycerol, even though such an inhibition was not obvious. Declercq *et al.* (1982) found that glucagon lowered the glycerol 3-phosphate concentration in hepatocytes. If the cyclic AMP analogues reduced the intracellular level of glycerol 3-phosphate, then the flux of [^3H]glycerol through this pool and labelling of diacylglycerol should have been enhanced. Since the incorporation of [^3H]glycerol into diacylglycerol was not stimulated in the presence of the analogues, diacylglycerol synthesis could have been inhibited, but the inhibition masked by the higher specific radioactivity of the precursors.

Palmitate reverses CPT-cAMP inhibition of triacylglycerol synthesis

Fatty acids have been shown to stimulate triacylglycerol synthesis in liver slices (Rose *et al.*, 1963; Nilsson & Schersten, 1969) and isolated hepatocytes (Sundler *et al.*, 1974; Haagsman & van Golde, 1981). We were curious to know if supplementation of hepatocytes with fatty acids could reverse the inhibition of triacylglycerol formation induced by cyclic AMP analogues. As shown in Fig. 2(a), 1 mM-palmitate completely abolished the difference in [^3H]glycerol entry into the triacylglycerol of control and 0.5 mM-CPT-cAMP-treated hepatocytes. The specific radioactivity of diacylglycerol was probably similar in 2 mM-oleate, and in 2 mM-oleate with 0.5 mM-CPT-cAMP-treated hepatocytes, since the pool size of diacylglycerol was also similar in these cells (Table 1). The amount of [^3H]glycerol in diacylglycerol was the same in control and CPT-cAMP-treated hepatocytes, although the presence of palmitate doubled the appearance of radiolabel into this pool in both

groups of cells (Fig. 2a). Therefore, it is likely the rates of the diacylglycerol acyltransferase-catalysed reaction and triacylglycerol synthesis in the presence of 1 mM-palmitate and in the presence of 1 mM-palmitate with 0.5 mM-CPT-cAMP-treated cells were the same.

The mechanism by which fatty acid reverses the cyclic AMP inhibition of net incorporation of [^3H]glycerol into triacylglycerol is obscure, although diacylglycerol acyltransferase activity using endogenous substrate is increased in microsomes from fatty acid-treated hepatocytes (Haagsman & van Golde, 1981). Increased availability of fatty acid produces corresponding increases in the intracellular concentrations of diacylglycerol and fatty acyl-CoA. If cyclic AMP analogues reduce the affinity of the acyltransferase for fatty acyl-CoA or diacylglycerol, then this might account for how fatty acid supplementation reverses the inhibition of triacylglycerol synthesis. Unfortunately, Haagsman *et al.* (1982) have not yet reported whether the K_m of the acyltransferase for palmitoyl-CoA or diacylglycerol is affected by phosphorylation, nor whether fatty acids directly stimulate the enzyme *in vitro*. The inhibitory effect of glucagon on diacylglycerol acyltransferase activity was not observed when saturating amounts of exogenous diacylglycerol were used in the enzyme assays (Haagsman *et al.*, 1981). In the present study, supplementation of hepatocytes with oleate doubled the size of the diacylglycerol pool (Table 1).

Oleate reverses cAMPS inhibition of phosphatidylcholine synthesis

We have shown that treatment of isolated rat hepatocytes with cyclic AMP analogues and cyclic AMP phosphodiesterase inhibitors reduces [$\text{Me-}^3\text{H}$]choline incorporation into phosphatidylcholine (Pelech *et al.*, 1981). In the present study, the influence of cAMPS on phosphatidylcholine synthesis was evaluated with a pulse-chase study (Fig. 3). To avert the problem of cAMPS altering the pool size of phosphocholine and the specific radioactivity of this precursor, the hepatocytes were prelabelled for 30 min with 15 μCi of [$\text{Me-}^3\text{H}$]choline. At the end of the pulse period, 90% of the label that had not been oxidized to betaine was associated with the phosphocholine pool of the cells. The inclusion of 0.5 mM-cAMPS in the chase medium reduced the rate of disappearance of label from phosphocholine and its appearance in phosphatidylcholine (Fig. 3). Since the specific radioactivity of [$\text{Me-}^3\text{H}$]phosphocholine was the same in all cells at the start of the chase, the reduced transfer of radioactivity from phosphocholine to phosphatidylcholine reflected an inhibition of the cytidyltransferase-catalysed reaction.

Although an inhibition of phosphatidylcholine

Table 1. *Effect of oleate and CPT-cAMP on the diacylglycerol pool size in isolated rat hepatocytes*

Monolayer cultures of rat hepatocytes were incubated for 1 h in the presence of 100 nM-insulin, 10 mg of bovine serum albumin/ml and various compounds. Diacylglycerol was estimated by the method of Schneider (1977). Each value represents the mean $\pm$ S.D. for four preparations.

Compound	Diacylglycerol (nmol/ 6×10^6 cells)
None	27.2 ± 1.5
Oleate (2 mM)	$44.4 \pm 2.5^*$
CPT-cAMP (0.5 mM)	24.9 ± 2.9
Oleate (2 mM) + CPT-cAMP (0.5 mM)	$46.1 \pm 3.0^*$

* $P < 0.01$ from a paired t test.

Fig. 2. The effect of palmitate on $[1(3)\text{-}^3\text{H}]\text{glycerol}$ incorporation into rat hepatocytes treated with 0.5 mM-CPT-cAMP. Cells (3×10^6) were incubated for 90 min in serum-free medium containing 100 nM-insulin and pulsed with $10 \mu\text{Ci}$ of $200 \mu\text{M}$ - $[1(3)\text{-}^3\text{H}]\text{glycerol}$ for 60 min in the presence of 0–2 mM-palmitate with or without 0.5 mM-CPT-cAMP. Symbols in (a): triacylglycerol, O and ●; diacylglycerol, Δ and ▲. Symbols in (b): phosphatidylcholine, ◇ and ◆; phosphatidylethanolamine, ▽ and ▼. Open symbols, controls; closed symbols, CPT-cAMP-treated. Each point represents the mean of three dishes, and for triacylglycerol the S.E.M. is indicated by bars. This experiment was repeated twice and with cAMPS instead of CPT-cAMP, all with similar results.

synthesis in hepatocytes with cyclic AMP analogues can be clearly demonstrated with $[\text{Me-}^3\text{H}]\text{choline}$ (Pelech *et al.*, 1981; Fig. 3), these cyclic AMP analogues did not appear to influence phospholipid formation when $[^3\text{H}]\text{glycerol}$ or $[^{14}\text{C}]\text{palmitate}$ was used to monitor synthesis (Fig. 2b; other results not shown). The reason for this is not clear. The $[^3\text{H}]\text{glycerol}$ and $[^{14}\text{C}]\text{palmitate}$ can be metabolized within the cell in numerous ways and this could complicate the interpretation of the results. In fact, only 10% of the $[^3\text{H}]\text{glycerol}$ and 20% of the $[^{14}\text{C}]\text{palmitate}$ appeared in phosphatidylcholine in these experiments. Furthermore, there may be specific pools of diacylglycerol that are utilized preferentially for phosphatidylcholine synthesis rather than for the synthesis of phosphatidylethanolamine or triacylglycerol. Cholinephosphotransferase and ethanolaminephosphotransferase are reported to have different specificities for various species of diacylglycerol (Holub, 1978; Morimoto & Kanoh, 1978). If the specific radioactivity of the diacylglycerol species destined for phosphatidylcholine synthesis were increased in CPT-cAMP- or cAMPS-treated hepatocytes, an inhibition of phosphatidylcholine by these cyclic AMP analogues would have been masked.

Fatty acids stimulate phosphatidylcholine synthesis in isolated rat hepatocytes (Sundler &

Åkesson, 1975; Fig. 3b) and we have recently demonstrated that the cytidyltransferase-catalysed step is accelerated in these cells (Pelech *et al.*, 1983). Since cytidyltransferase is also subject to control by protein phosphorylation (Pelech & Vance, 1982), the influence of 0–4 mM-oleate and 0.5 mM-cAMPS on phosphatidylcholine synthesis was investigated (Table 2). Oleate stimulated this synthesis in 0.5 mM-cyclic AMP analogue-inhibited and control cells. With 3 mM-oleate in the hepatocyte medium, the rate of phosphatidylcholine synthesis in cyclic AMP analogue-treated and untreated cells was almost the same. Oleate at 2 mM was insufficient to abolish the inhibitory effects of cAMPS or CPT-cAMP as approx. 16% less phosphatidylcholine was formed during the chase period compared with that in cells that were exposed to the fatty acid but not the analogues (results not shown).

Recent studies in our laboratory have indicated that conditions that favour protein phosphorylation reduce the binding of cytidyltransferase to microsomes (S. Pelech & D. Vance, unpublished work). Apparently this ambiguous enzyme (Wilson, 1978), which is rapidly and reversibly interconverted between soluble and membrane-bound forms, is most active when it is membrane-associated (Sleight & Kent, 1980, 1983a,b; Weinhold *et al.*, 1981; Pritchard *et al.*, 1982).

Fig. 3. The effect of cAMPs on the disappearance of $[Me-^3H]$ choline from cellular phosphocholine and accumulation into phosphatidylcholine

Cultured rat hepatocytes in 60 mm dishes were pulse-labelled for 30 min with $15 \mu\text{Ci}$ of $[Me-^3H]$ choline. The cells were washed and fresh medium with or without 0.5 mM -cAMPs was added. At various times, the cells and media were collected, and the radioactivity was measured in (a) phosphocholine (control, ∇ , treated, $\blacktriangledown$) and in (b) phosphatidylcholine (control, Δ ; treated, $\blacktriangle$). Each point represents the mean of three dishes, and the S.E.M. is indicated by bars.

Table 2. Effect of oleate on cyclic AMP analogue inhibition of phosphatidylcholine biosynthesis

Isolated rat hepatocytes were prelabelled for 30 min with $12.5 \mu\text{Ci}$ of $[Me-^3H]$ choline and chased for an additional 45 min with non-radioactive medium that contained 0.5 mM -CPT-cAMP or 0.5 mM -cAMPs, in the absence or in the presence of 3 mM -oleate. Each value is the mean ($\pm$ S.D.) of three dishes. The incorporation into phosphatidylcholine during the pulse period $[(1.63 \pm 0.12) \times 10^5 \text{ d.p.m./dish}]$ has been subtracted. This experiment was reproduced with three preparations of hepatocytes.

Compound	$10^{-5} \times$ Incorporation into phosphatidylcholine (d.p.m./dish)	
	– Oleate	+ Oleate
Control	2.97 ± 0.12	6.78 ± 0.30
CPT-cAMP	1.62 ± 0.35	6.39 ± 0.44
cAMPs	2.22 ± 0.14	6.37 ± 0.16

Typically 80% of the cytidylyltransferase is normally in a soluble form in rat liver. We have reported that fatty acids and fatty acyl-CoA promote the translocation of the cytidylyltransferase to the endoplasmic reticulum (Pelech *et al.*, 1983). Hence in the present study, reversal of the inhibition of phosphatidylcholine synthesis by fatty acids could

have been mediated by a redistribution of the enzyme back to the endoplasmic reticulum.

This supposition was verified in studies that exploited the ability of digitonin to initiate the release of the cytosolic contents of hepatocytes into the medium (MacKall *et al.*, 1979). When cells that had been pre-incubated for 1 h with 0.5 mM -CPT-cAMP were treated with digitonin for 2 min, 22% more ($P < 0.05$) cytidylyltransferase was leaked into the medium than observed from untreated cells (Fig. 4). However, in the presence of oleate (3 mM) and CPT-cAMP (0.5 mM), or the fatty acid alone, less than one-fifth ($P < 0.01$) as much cytidylyltransferase appeared in the medium as compared with untreated cells. The reduced rate of cytidylyltransferase release from the cells exposed to digitonin was probably due to the conversion of the enzyme into the membrane-bound form (Pelech *et al.*, 1983).

These findings may account for fluctuations in the cytosolic and microsomal activities of cytidylyltransferase in rat liver after starvation for 24 and 48 h. Starvation resulted in a doubling of the microsomal enzyme activity, with a corresponding reduction of the cytosolic enzyme activity (S. Pelech, C. Wong & D. Vance, unpublished work). Although in starved animals, glucagon initiates a cascade that presumably results in phosphorylation and translocation of the cytidylyltransferase to the

Fig. 4. Digitonin-mediated release of cytidylyltransferase from oleate- and CPT-cAMP-treated hepatocytes

Rat hepatocytes in 60 mm dishes (2×10^6 cells) were incubated for 1 h in serum-free medium containing 100 nM-insulin, and for an additional 1 h in the absence (●) or in the presence of 0.5 mM-CPT-cAMP (△), 3 mM-oleate (□) or 0.5 mM-CPT-cAMP and 3 mM-oleate (▽). The cell medium was replaced with 1.5 ml of ice-cold digitonin (0.5 mg/ml) and the incubation continued on ice-cold metal trays for up to 4 min. Cytidylyltransferase activity in the digitonin extract was subsequently determined in the presence of 150 µg of total rat liver phospholipid and 100 µM-oleate. Each point represents the mean of three dishes and the standard error is indicated by the bars. This experiment was repeated once with similar results.

cytosol, a redistribution of the enzyme to the endoplasmic reticulum is favoured instead. This probably arises because fatty acids mobilized from adipose tissue and from lipolysis of hepatic triacylglycerol (Debeer *et al.*, 1982) promote the conversion of even the apparently phosphorylated cytidylyltransferase to the membrane-bound form (Pelech *et al.*, 1983).

The present paper has shown that the inhibitory effects of cyclic AMP analogues on the synthesis of phosphatidylcholine and triacylglycerol can be removed by increasing concentrations of fatty acids. *In vivo*, the fatty acid concentration in blood (normally about 0.2 mM) is elevated during stress conditions, when the intracellular level of cyclic AMP in liver is also increased. Cyclic AMP may be

involved in modifying the kinetic properties of a number of lipogenic enzymes so that oxidation is the major fate of imported fatty acids, especially when the fatty acid concentration is low. However, when the supply of fatty acid is abundant, as during starvation, diabetes and stress, esterification into glycerolipid can be enhanced. This would prevent an accumulation of potentially toxic non-esterified fatty acids and acyl-CoA esters in the liver. The subsequent secretion of triacylglycerols in very-low-density lipoproteins also provides muscle tissue with a source of energy (Brindley, 1981; Lawson *et al.*, 1982).

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