Regulation of the translocation of phosphatidate phosphohydrolase between the cytosol and the endoplasmic reticulum of rat liver

Effects of unsaturated fatty acids, spermine, nucleotides, albumin and chlorpromazine

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1. The translocation of phosphatidate phosphohydrolase between the cytosol and the microsomal membranes was investigated by using a cell-free system from rat liver. 2. Linoleate, α -linolenate, arachidonate and eicosapentenoate promoted the translocation to membranes with a similar potency to that of oleate. 3. The phosphohydrolase that associated with the membranes in the presence of [14 C]oleate or 1 mM-spermine coincided on Percoll gradients with the peak of rotenone-insensitive NADH-cytochrome *c* reductase, and in the former case with a peak of 14 C. 4. Microsomal membranes were enriched with the phosphohydrolase activity by incubation with [14 C]oleate or spermine and then incubated with albumin. The phosphohydrolase activity was displaced from the membranes by albumin, and this paralleled the removal of [14 C]oleate from the membranes when this acid was present. 5. Chlorpromazine also displaced phosphatidate phosphohydrolase from the membranes, but it did not displace [14 C]oleate. 6. The effects of spermine in promoting the association of the phosphohydrolase with the membranes was inhibited by ATP, GTP, CTP, AMP and phosphate. ATP at the same concentration did not antagonize the translocating effect of oleate. 7. From these results and previous work, it was concluded that the binding of long-chain fatty acids and their CoA esters to the endoplasmic reticulum acts as a signal for more phosphatidate phosphohydrolase to associate with these membranes and thereby to enhance the synthesis of glycerolipids, especially triacylglycerol. The translocation of the phosphohydrolase probably depends on the increased negative charge on the membranes, which could also be donated by the accumulation of phosphatidate. Chlorpromazine could oppose the translocation by donating a positive charge to the membranes.

INTRODUCTION

The Mg^{2+} -stimulated PAP activity in most mammalian cells is located in both the cytosol and the endoplasmic reticulum (Brindley & Sturton, 1982). The relative proportion of PAP activity in these two locations can be modified by altering the ionic strength of the homogenization medium (Moller & Hough, 1982; Walton & Possmayer, 1984). In the cell the phosphatidate that serves as the substrate for PAP is generated on the membranes of mitochondria and the endoplasmic reticulum. Consequently, the cytosolic PAP must associate with these membranes if it is to be metabolically functional. It has been shown that the cytosolic PAP of intact rat hepatocytes can translocate into the membrane-associated compartment when the concentration of oleate in the incubation medium is increased (Cascales *et al.*, 1984; Butterwith *et al.*, 1984b). This translocation can also be demonstrated in a cell-free system by incubation with palmitate and oleate or with their CoA esters (Martin-Sanz *et al.*, 1984). Spermine promoted the action of oleate in this respect (Martin-Sanz *et al.*, 1985).

It was therefore proposed that the cytosolic PAP represents an inactive reservoir of activity that becomes metabolically functional when it translocates to the membrane-associated compartment (Brindley, 1984).

The effects of long-chain fatty acids and their CoA esters in facilitating this phenomenon makes physiological sense, since it enables cells to match their rates of triacylglycerol synthesis to the net rate of fatty acid supply that they encounter. This helps to control the extent to which fatty acids and their CoA esters can accumulate, and thus it protects cells against the potentially toxic effects of these compounds. The ability of PAP to attach to the membranes appears to be modified by the acute effects of hormones. The addition of a cyclic AMP analogue to hepatocytes displaced PAP from the membranes at low fatty acid concentrations. However, this displacement was not observed when 1 mM-oleate was added to the hepatocytes (Butterwith *et al.*, 1984b). The changes in membrane-bound PAP activity were paralleled in the rates of triacylglycerol synthesis. This was decreased by cyclic AMP analogues at low fatty acid concentrations, and the inhibition was overcome by adding fatty acid (Pelech *et al.*, 1983). Glucagon can also produce similar effects on the subcellular distribution of PAP to those of the cyclic AMP analogues. By contrast, insulin appears to promote the association of PAP with the membranes at low fatty acid concentrations (Pittner *et al.*, 1985). In adipocytes the addition of lipolytic agents caused a greater proportion of the PAP to become associated with the microsomal fraction, and there was

Abbreviation used: PAP, phosphatidate phosphohydrolase (EC 3.1.3.4).

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a fall in the cytosolic activity (Moller *et al.*, 1980). These results can also now be possibly explained by a fatty acid-induced translocation of PAP.

This combined evidence establishes that PAP can be classified as an 'ambiquitous' enzyme (Wilson, 1980). It exists in different compartments of the cell, and its translocation between the compartments helps to regulate metabolism. The present experiments were performed to investigate in greater detail how the translocation of PAP can be regulated. The experiments were performed by using the cell-free system that was previously established (Martin-Sanz *et al.*, 1984, 1985).

EXPERIMENTAL

Animals and materials

The sources of the rats and most of the materials have been described (Cascales *et al.*, 1984; Martin-Sanz *et al.*, 1984). The fatty acids, nucleotides and Percoll were obtained from Sigma (London) Chemical Co., Poole, Dorset, U.K. [^{14}C]Oleic acid (50 Ci/mol) was purchased from Amersham International, Amersham, Bucks., U.K. Chlorpromazine was a gift from May and Baker, Dagenham, Essex, U.K.

Preparation and treatment of subcellular fractions

Subcellular fractions were prepared from the livers of male Wistar rats by the methods described by Martin-Sanz *et al.* (1984). In some experiments 20 mM-Hepes was added to the sucrose medium, and the pH was adjusted to 7.4 with KOH before the homogenization rather than after the preparation of subcellular fractions.

Solutions of the fatty acids (in a 20% molar excess of KOH), spermine or nucleotides were added as indicated in a final volume of 1.5 ml to 20–25 mg of protein from the supernatant obtained after centrifugation for 10 min at 18000 *g*. The mixtures were incubated for 10 min at 37 °C and then cooled in ice (Martin-Sanz *et al.*, 1984). In some experiments fatty-acid-poor bovine serum albumin or chlorpromazine was then added, and the samples were incubated for a further 10 min at 37 °C. The microsomal and soluble fractions were collected after centrifugation at 90000 *g* ($r_{\text{av.}} = 6.3$ cm) for 90 min at 4 °C, and the microsomal fraction was resuspended in 0.25 M-sucrose containing 0.2 mM-dithiothreitol and 20 mM-Hepes buffer, pH 7.4. Fractions were analysed for PAP activity and [^{14}C]oleate where appropriate.

Separation of cytosolic and membrane-bound proteins on Percoll gradients

Samples of the microsomal fraction and the particle-free supernatant were prepared (Martin-Sanz *et al.*, 1984), and a part of these fractions was recombined in the same proportions as in the supernatant from which they were derived. Portions of the particle-free supernatant, or the recombined microsomal fraction and the particle-free supernatant, were then incubated for 10 min at 37 °C in the presence or absence of oleate (12.5 mCi/mol) and spermine. Samples (1 ml) of these incubations were cooled to 4 °C and layered over 10 ml of Percoll (density 1.07 g/ml) suspended in 0.25 M-sucrose containing 0.2 mM-dithiothreitol and 20 mM-Hepes buffer, pH 7.4. The tubes were centrifuged in a 'soft start centrifuge' at 63000 *g* ($r_{\text{av.}} = 5.82$ cm) for 30 min at 4 °C. Fractions (1 ml) were collected from the top of each tube by using

a peristaltic pump and a fraction collector. The density of each fraction was calculated by weighing 0.1 ml.

Analytical methods

The methods used for the determinations were as follows: for PAP (Butterwith *et al.*, 1984a), which was assayed in the presence of 5 mM-MgCl₂ and a mixed emulsion of 0.4 mM-phosphatidylcholine and 0.6 mM-phosphatidate (0.4 Ci/mol) labelled with [^3H]palmitate; for lactate dehydrogenase (Saggerson & Greenbaum, 1969); for the rotenone-insensitive NADH-cytochrome *c* reductase (Sottocasa *et al.*, 1967); and for protein (Martin-Sanz *et al.*, 1984). [^{14}C]Oleate was determined by liquid-scintillation counting.

RESULTS

Effects of long-chain polyunsaturated fatty acids on the translocation of PAP to microsomal membranes

In previous experiments with a cell-free preparation that contained the cytosol and microsomal membranes from rat liver, palmitate and oleate were used to translocate PAP on to the microsomal membranes. Oleate was more effective than palmitate in this respect (Martin-Sanz *et al.*, 1984). Table 1 shows that several polyunsaturated fatty acids have a similar effect to that of oleate. The relative distribution of PAP in the microsomal membranes for the control incubations was higher than that reported previously by Martin-Sanz *et al.* (1984) and those in Table 2. This could be related to the inclusion of 20 mM-Hepes buffer, pH 7.4, in the sucrose medium used for the homogenization in the experiments shown in Table 1.

Effects of oleate and spermine on the translocation of PAP to the endoplasmic reticulum

In these experiments Percoll gradients were used to determine the interaction of oleate and PAP with the membranes of the endoplasmic reticulum. The microsomal fraction was recombined with the cytosolic fraction in the gradients shown in Figs. 1(a)–1(d), and the mixture was incubated with combinations of [^{14}C]oleate and spermine. The gradients shown in Figs. 1(e)–1(h) were established as control experiments to investigate the requirement for the microsomal membranes. The activity of the endoplasmic reticulum marker in the cytosolic fraction was less than 1% of that in the recombined microsomal and cytosolic fraction (see differences in scale for this enzyme).

Some 90% of the PAP activity in the mixed microsomal and cytosolic fraction, which was incubated without further additions, was recovered in the top four fractions, and the PAP activity coincided with that of lactate dehydrogenase (Fig. 1a). The addition of 350 μM -oleate caused about 25% of the PAP activity to translocate to the region in which the majority of the NADH-cytochrome *c* reductase was found. About 72% of the [^{14}C]oleate coincided with the NADH-cytochrome *c* reductase activity (Fig. 1b), and this probably explains why the mean density of the endoplasmic-reticulum membranes had decreased compared with that in Fig. 1(a). The use of 750 μM -oleate in the incubations caused 90% of the PAP activity to coincide with the endoplasmic-reticulum marker (results not shown). By contrast, oleate did not

Table 1. Effects of long-chain polyunsaturated fatty acids on the translocation of PAP between the cytosol and the microsomal membranes

Rat livers were homogenized in 0.25 M-sucrose containing 0.2 mM-dithiothreitol and 20 mM-Hepes, adjusted to pH 7.4 with KOH, and a supernatant was obtained after centrifuging for 10 min at 18000 *g*. This was incubated for 10 min at 37 °C with 500 μ M of the fatty acids as indicated. The membranes and the cytosol were then separated by centrifugation (see the Experimental section). The relative PAP activities are quoted as means $\pm$ S.D. where there are more than two independent experiments, or $\pm$ ranges for two independent experiments, as shown in parentheses. The mean PAP activity of the controls was 0.7 ± 0.05 nmol of diacylglycerol formed/min per mg of protein.

Additions	Relative distribution of PAP activity (%)		Total PAP activity relative to control
	Soluble fraction	Microsomal fraction	
None (control)	75 $\pm$ 7	25 $\pm$ 7	1.00 (7)
Oleate	17 $\pm$ 7	83 $\pm$ 7	1.15 $\pm$ 0.24 (6)
Linoleate	21 $\pm$ 3	79 $\pm$ 3	1.06 $\pm$ 0.06 (2)
α -Linolenate	22 $\pm$ 1	78 $\pm$ 1	1.10 $\pm$ 0.08 (2)
Arachidonate	20 $\pm$ 2	80 $\pm$ 2	1.11 $\pm$ 0.11 (2)
Eicosapentenoate	40 $\pm$ 21	60 $\pm$ 21	1.10 $\pm$ 0.04 (2)

cause any significant translocation of the cytosolic marker lactate dehydrogenase.

The addition of 1 mM-spermine aggregated the endoplasmic-reticulum membranes, and a tighter band of membranes was produced (Fig. 1c). About 30% of the PAP activity was coincident with these membranes. The addition of 350 μ M-oleate together with 1 mM-spermine caused more than 80% of the PAP activity to translocate to the position occupied by the endoplasmic-reticulum marker. These two enzyme activities were also coincident with the majority (about 73%) of the [14 C]oleate that was added.

Control experiments with the particle-free supernatant demonstrated that the addition of either 350 μ M-oleate or 1 mM-spermine did not cause the appearance of PAP in the region of tube 5 from the gradient, and its activity coincided with that of lactate dehydrogenase (Figs. 1e–1g). However, in previous work with spermine about 11% of the PAP activity was pelleted by centrifugation (Martin-Sanz *et al.*, 1985). This small amount of aggregated PAP may not have been detected if it occurred at the bottom of the Percoll gradient (Fig. 1g). The combined addition of oleate and spermine caused about 42% of the PAP activity to migrate down the gradient along with 55% of the [14 C]oleate and 41% of the residual NADH-cytochrome *c* reductase activity (Fig. 1h). In the absence of membrane remnants, spermine in the absence or presence of 10 mg of albumin/ml did not promote the migration of [14 C]oleate down Percoll gradients (results not shown).

Displacement of PAP from the microsomal membranes by albumin and chlorpromazine

The results in Fig. 1 demonstrate that the translocation of PAP to the membranes is accompanied by the association of [14 C]oleate with these membranes. This was confirmed by further analysis (Brindley & Bowley, 1975). The 14 C was quantitatively extracted and more than 92% of the radioactivity co-chromatographed on t.l.c. with authentic oleate. It should therefore be possible to reverse the translocation by adding albumin to decrease the concentration of membrane-bound fatty acid. This is illustrated in Fig. 2, in which microsomal membranes

were preincubated with [14 C]oleate in the presence of the cytosol in order to translocate PAP to them. The membranes were then incubated with various concentrations of fatty-acid-poor bovine serum albumin and then re-isolated. The albumin decreased the [14 C]oleate that was bound to the membranes and caused a parallel displacement of PAP activity into the cytosol. The same effect was seen when the membranes had been preincubated with both spermine and oleate (Fig. 3). Incubation with albumin also decreased the activity of the membrane-bound PAP in the membranes that had been preincubated with spermine alone or without additions. At 6.7 mg of albumin/ml the activity of the membrane-bound PAP for all four preincubation conditions approached the same value.

Chlorpromazine also displaces PAP from the membranes, and this effect is also shown in Fig. 2(b). However, this did not result from the displacement of [14 C]oleate. In fact, slightly more [14 C]oleate appeared to associate with the membranes as the chlorpromazine concentration was increased.

Effects of various nucleotides, phosphate, Mg^{2+} and Ca^{2+} on the translocation of PAP

ATP is known to reverse the stimulation that is produced by spermine in the synthesis of glycerolipids *in vitro* (Jamdar, 1977). This effect was thought to be caused by the chelation of the polyamine. The addition of various nucleotides to the incubations used to study the translocation of PAP antagonized the effects of 1 mM-spermine in the absence of oleate (Table 2, rows B–F). ATP appeared to be particularly effective in this respect. The spermine-induced translocation was also abolished by potassium phosphate buffer, pH 7.4 (Table 2, rows G–I). Approx. 44 mM-phosphate is required to produce the same effect as 2.5 mM-ATP (results not shown). The addition of Mg^{2+} or Ca^{2+} at an equimolar concentration to ATP did not reverse its inhibitory effect on the spermine-induced translocation (Table 2, rows J and K).

ATP also antagonized the effects of spermine in promoting the translocation of PAP to membranes in the presence of oleate (Table 2, rows Q and T). This antagonism was also not reversed by Mg^{2+} at concen-

Fig. 1. Effects of spermine and oleate on the association of cytosolic PAP with the endoplasmic reticulum of rat liver

Samples of a combined microsomal and particle-free supernatant fraction (a-d) or the particle-free supernatant fraction alone (e-h) were incubated with oleate and spermine as indicated, or in their absence (control). The fractions were then separated on Percoll gradients (see the Experimental section). The graphs show the distribution of [14C]oleate (▼), density (---) and the activities of PAP (○), rotenone-insensitive NADH-cytochrome c reductase (■) and lactate dehydrogenase (△). Similar results were obtained in five further independent experiments with oleate alone, or one further experiment involving spermine.

Fig. 2. Effects of albumin and chlorpromazine on the oleate-induced translocation of PAP between the cytosolic and microsomal fractions of rat liver

The supernatant obtained after centrifuging homogenates from rat liver for 10 min at 18000 g was incubated for 10 min with 0.5 mM- $[^{14}C]$ oleate (12.5 mCi/mol) in order to associate PAP with the membranes. Albumin (a) or chlorpromazine (b) was then added, and the incubation was continued for a further 10 min, whereupon the cytosolic fraction was separated from the microsomal membranes by centrifugation (see the Experimental section). The relative distributions of the ^{14}C ($\blacktriangle$, $\bullet$) and PAP ($\triangle$, $\circ$) are shown in the microsomal ($\triangle$, $\blacktriangle$) and cytosolic ($\circ$, $\bullet$) fractions. Similar results were obtained in a further independent experiment.

Fig. 3. Effects of albumin on the oleate- and spermine-induced translocation of PAP to the microsomal membranes of rat liver

The supernatant obtained after centrifuging homogenates of rat liver for 10 min at 18000 g was incubated for 10 min with 0.5 mM- $[^{14}C]$ oleate (12.5 mCi/mol) and 1 mM-spermine as indicated to associate PAP with the membranes. Albumin was added and the incubations were continued for a further 10 min, whereupon the cytosolic fraction was separated from the microsomal membranes by centrifugation (see the Experimental section). The relative distributions of PAP (a) and $[^{14}C]$ oleate, where appropriate (b), are shown for incubations that contained: oleate and spermine ($\triangle$), oleate ($\circ$), spermine ($\blacksquare$), or no additions ($\bullet$). Similar results were obtained in two further independent experiments.

trations between 2.5 and 20 mM (Table 2, rows U–X). We had thought that the chelation of Mg^{2+} with ATP might lessen the interaction with spermine and thus decrease the effects of ATP on the translocation.

The various nucleotides had no significant effects on the distribution of PAP between the membranes and the cytosol when they were added in the absence of spermine (results not shown). ATP did not antagonize the translocation of PAP to the membranes that was induced by oleate, but the percentage of the PAP that bound to the membranes when oleate and spermine was added together was decreased by ATP (Table 2).

Ca^{2+} and Mg^{2+} at concentrations of 0.1–2.5 mM and EGTA or EDTA at 1 mM did not significantly alter the relative distribution of PAP between the membrane-

bound and cytosolic fractions, either alone or in the presence of oleate and/or spermine (results not shown).

Phosphate buffer increased the association of PAP with the microsomal membranes in the absence (Table 2, rows L and M) or presence of oleate (Table 2, row O). A similar stimulation in the interaction of PAP with the artificial membranes of phosphatidylcholine and phosphatidate that are used as a substrate in the enzyme assay could also explain why phosphate buffer also increases PAP activity (Butterwith *et al.*, 1984a). Curiously, the combination of phosphate buffer with spermine in the absence (Table 2, rows G–I), or presence of oleate (Table 2, row R), caused less PAP to associate with the membranes. We cannot adequately explain why spermine and phosphate, which both individually promote the translocation of PAP to the

Table 2. Effects of spermine, oleate, Mg^{2+} , Ca^{2+} and various nucleotides on the translocation of PAP between the cytosol and the microsomal membranes

Rat liver homogenates were prepared in 0.25 M-sucrose that contained 0.2 mM-dithiothreitol and which was adjusted to pH 7.4 with $KHCO_3$ (Martin-Sanz *et al.*, 1984). A supernatant was prepared by centrifugation for 10 min at 18000 g, and Hepes (pH 7.4) was added to a final concentration of 20 mM. The supernatant was incubated for 10 min at 37 °C with 1 mM-spermine, 500 μ M-oleate, 20–100 mM-potassium phosphate buffer, pH 7.4, 2.5–20 mM- $MgCl_2$, or 2.5 mM- Ca^{2+} , or the various nucleotides (see the Experimental section). The relative activities of PAP are quoted as means $\pm$ S.D. where there are more than two experiments, or means $\pm$ ranges for two independent experiments as shown in parentheses. The mean PAP activity of the controls was 2.08 ± 0.82 nmol of diacylglycerol formed/min per mg of protein.

Additions	Relative distribution of PAP activity (%)		
	Soluble fraction	Microsomal fraction	Total PAP activity relative to control
(A) None (control)	92 $\pm$ 3	8 $\pm$ 3	1.00 (17)
(B) + spermine	67 $\pm$ 9	33 $\pm$ 9	0.74 $\pm$ 0.16 (13)
(C) + spermine + ATP	90 $\pm$ 5	10 $\pm$ 5	0.93 $\pm$ 0.06 (5)
(D) + spermine + GTP	79 $\pm$ 4	21 $\pm$ 4	0.87 $\pm$ 0.14 (2)
(E) + spermine + CTP	82 $\pm$ 2	18 $\pm$ 2	0.89 $\pm$ 0.09 (2)
(F) + spermine + AMP	71 $\pm$ 4	29 $\pm$ 4	0.82 $\pm$ 0.10 (2)
(G) + spermine + phosphate (20 mM)	82 $\pm$ 7	18 $\pm$ 7	1.05 $\pm$ 0.16 (4)
(H) + spermine + phosphate (50 mM)	91 $\pm$ 4	9 $\pm$ 4	0.86 $\pm$ 0.05 (3)
(I) + spermine + phosphate (100 mM)	95 $\pm$ 3	5 $\pm$ 3	1.04 $\pm$ 0.13 (2)
(J) + spermine + ATP + Mg^{2+}	90 $\pm$ 1	10 $\pm$ 1	0.90 $\pm$ 0.03 (2)
(K) + spermine + ATP + Ca^{2+}	94 $\pm$ 1	6 $\pm$ 1	1.00 $\pm$ 0.03 (2)
(L) + phosphate (20 mM)	82 $\pm$ 7	18 $\pm$ 7	1.15 $\pm$ 0.13 (2)
(M) + phosphate (50 mM)	77 $\pm$ 10	23 $\pm$ 10	0.92 $\pm$ 0.07 (4)
(N) + oleate	53 $\pm$ 13	47 $\pm$ 13	0.87 $\pm$ 0.13 (13)
(O) + oleate + phosphate (50 mM)	30 $\pm$ 18	70 $\pm$ 18	1.14 $\pm$ 0.05 (4)
(P) + oleate + ATP	43 $\pm$ 6	57 $\pm$ 6	0.86 $\pm$ 0.02 (2)
(Q) + oleate + spermine	20 $\pm$ 11	80 $\pm$ 11	0.69 $\pm$ 0.10 (10)
(R) + oleate + spermine + phosphate (50 mM)	43 $\pm$ 8	57 $\pm$ 8	1.10 $\pm$ 0.13 (2)
(S) + oleate + spermine + Mg^{2+} (2.5 mM)	21 $\pm$ 2	79 $\pm$ 2	0.56 $\pm$ 0.04 (2)
(T) + oleate + spermine + ATP	32 $\pm$ 8	68 $\pm$ 8	0.85 $\pm$ 0.26 (5)
(U) + oleate + spermine + ATP + Mg^{2+} (2.5 mM)	29 $\pm$ 7	71 $\pm$ 7	0.81 $\pm$ 0.10 (4)
(V) + oleate + spermine + ATP + Mg^{2+} (5 mM)	24 $\pm$ 3	77 $\pm$ 3	0.82 $\pm$ 0.10 (2)
(W) + oleate + spermine + ATP + Mg^{2+} (10 mM)	31 $\pm$ 7	69 $\pm$ 7	0.67 $\pm$ 0.04 (2)
(X) + oleate + spermine + ATP + Mg^{2+} (20 mM)	32 $\pm$ 5	68 $\pm$ 5	0.83 $\pm$ 0.14 (2)

membranes, should have no significant effect when added together in the absence of oleate (Table 2, rows G–I). Although phosphate could complex with the 1 mM-spermine, there should still have been a large excess of phosphate capable of promoting translocation, especially when its concentration was 100 mM. In the presence of oleate (Table 2, row R), 50 mM-phosphate buffer partly reversed the effects of spermine in promoting the translocation of PAP to the membranes.

DISCUSSION

The present results demonstrate that oleate can cause PAP to translocate from the cytosol and associate with microsomal membranes. In fact, the translocated PAP co-migrates on Percoll gradients with the endoplasmic reticulum (Fig. 1). We have not absolutely proved that the association is exclusively with endoplasmic-reticulum membranes and that there is no binding to other membranes of the microsomal fraction. However, the association of PAP with the endoplasmic reticulum is compatible with its known subcellular distribution, which has been described in a variety of tissues (Brindley & Sturton, 1982). This association also makes metabolic

sense in that its substrate, phosphatidate, is generated in the endoplasmic reticulum.

Oleate appears to cause PAP to associate with the endoplasmic reticulum because it also binds to these membranes (Fig. 1). In the absence of the membrane fraction there is no significant migration of PAP down the gradient (Fig. 1f). The removal of oleate from the membranes that occurs when albumin is added causes a parallel displacement of PAP from the membranes (Figs. 2 and 3). Similarly, albumin has been shown to displace CTP:phosphocholine cytidyltransferase (EC 2.7.7.15) from microsomal membranes derived from lung (Weinhold *et al.*, 1984). This enzyme is also activated by a fatty-acid-induced translocation from the cytosol to the endoplasmic reticulum (Pelech & Vance, 1984).

PAP can also be translocated to the endoplasmic reticulum by a variety of polyunsaturated fatty acids (Table 1) and to a lesser extent by palmitate (Martin-Sanz *et al.*, 1984). Palmitoyl-CoA and oleoyl-CoA are more effective than their unesterified acids in this respect. The inability of octanoate and octanoyl-CoA to cause the translocation (Martin-Sanz *et al.*, 1984) probably results from their lower lipid and membrane solubilities. The translocation of PAP to the membranes was also

increased when they were enriched with phosphatidate (R. Hopewell & D. N. Brindley, unpublished work).

The association of long-chain fatty acids, their acyl-CoA esters and phosphatidate with the membranes should increase the negative charge on their surfaces, and this might be the signal for the attachment of PAP. We therefore thought that this attraction might be modified by the presence of bivalent cations such as Mg^{2+} or Ca^{2+} . However, these cations did not alter the ability of oleate to promote the translocation. By contrast, the amphiphilic cation chlorpromazine did antagonize the effect of oleate on the translocation without displacing the acid from the membranes (Fig. 2). Chlorpromazine is known to bind tightly to membranes, and in so doing it increases the positive charge on their surface. This may in fact explain why slightly more [^{14}C]oleate associated with the membranes as the concentration of chlorpromazine was increased (Fig. 2). Chlorpromazine also reverses the spermine-induced translocation of PAP to the membranes (R. Hopewell & D. N. Brindley, unpublished work).

The results in Fig. 1 and Table 2 confirm previous work showing that spermine can increase the activity of PAP that is associated with microsomal membranes from adipose tissue (Moller & Hough, 1982; Jamdar & Osborne, 1983a) and liver (Martin-Sanz *et al.*, 1985). The effect was not simply caused by the aggregation of PAP (Martin-Sanz *et al.*, 1985), and there was no significant migration of PAP activity down the gradients when spermine was added alone to the particle-free supernatant (Fig. 1f). However, in the presence of oleate, there was a peak of PAP activity in fraction 5 that coincided with a peak of [^{14}C]oleate and of the residual NADH-cytochrome *c* reductase activity (Fig. 1h). It is therefore concluded that spermine promotes the association of hydrophobic proteins with lipids and membranes. It is not replacing a requirement for Mg^{2+} for the translocation, since this bivalent cation was inactive in this respect (Table 2).

Spermine is known to form complexes with acyl-CoA esters, and this probably accounts for its stimulation of the activities of glycerol phosphate acyltransferase (Jamdar, 1979; Bates & Saggerson, 1981) and diacylglycerol acyltransferase (Jamdar, 1977; Jamdar & Osborne, 1983b). The translocation of more PAP to the microsomal membranes could be a further factor that could enhance triacylglycerol synthesis *in vitro* in the presence of spermine. The ability of spermine to interact with acyl-CoA esters and to aggregate microsomal membranes is inhibited by ATP (Jamdar, 1979). This same phenomenon probably accounts for the effects of nucleotides and phosphate in preventing the spermine-induced translocation of PAP to the microsomal membranes (Table 2). This inhibition was not seen when oleate alone was used to promote the translocation.

The role of polyamines in controlling PAP activity and glycerolipid synthesis *in vivo* is not well understood. The reversal of the spermine-induced translocation of PAP activity to membranes by nucleotides and phosphate brings into question the extent to which polyamines might affect the translocation in intact cells. Conversely, there are several indirect lines of evidence that suggest that polyamines could modify glycerolipid synthesis. These include effects on Ca^{2+} metabolism and the similarities

between the long-term control of PAP activity and that of ornithine decarboxylase (see Martin-Sanz *et al.*, 1985, for details). Furthermore, a stimulation of glycerolipid synthesis would be compatible with the growth-promoting effects of polyamines.

The present results provide further information concerning the mechanisms that control the translocation of PAP from the cytosol to the membranes of the endoplasmic reticulum, where glycerolipid synthesis is known to occur. This is thought to represent a functional activation of a reserve capacity of enzyme activity. It is hoped that a knowledge of the factors that control the translocation will provide a further level at which to understand the physiological and pharmacological mechanisms that can be used to regulate glycerolipid synthesis.

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