Spermine promotes the translocation of phosphatidate phosphohydrolase from the cytosol to the microsomal fraction of rat liver and it enhances the effects of oleate in this respect

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Spermine (0.5–2 mM) promoted the translocation of phosphatidate phosphohydrolase from the soluble to the microsomal fraction in a cell-free system derived from rat liver. By contrast, spermidine (1 mM) and putrescine (1 mM) had no significant effect on the translocation when added alone. Spermine, and to a lesser extent, spermidine, enhanced the translocating action of oleate and increased its effectiveness in transferring the phosphohydrolase from the soluble ot the microsomal fraction. It is proposed that the phosphohydrolase becomes metabolically active when it combines with membranes and that polyamines might help to regulate this interaction. This could facilitate the action of fatty acids and enable cells to increase their capacity for triacylglycerol synthesis to match an increased availability of fatty acids.

Fatty acid *L-α-Phosphatidate phosphohydrolase* *Polyamine* *Translocation* *Triacylglycerol synthesis*

1. INTRODUCTION

Phosphatidate phosphohydrolase is thought to be important in regulating triacylglycerol synthesis in the liver, particularly in metabolic stress [1]. Increases in the enzyme activity appear to parallel the increased triacylglycerol synthesis seen when the liver is confronted with a high fatty acid supply [2,3].

A reserve capacity of the phosphohydrolase appears to reside in the cytosolic form of the enzyme that can be activated by translocation onto the membranes that synthesize triacylglycerols. This translocation takes place progressively when hepatocytes are exposed to increasing concentrations of oleate [3].

Recently we were able to demonstrate that long-chain fatty acids and their acyl-CoA esters promote the translocation of phosphatidate phosphohydrolase from the soluble to the microsomal fraction in cell-free preparations of rat liver [4]. This provides a model system with which to investigate the action of other compounds that might affect the translocation. It seemed likely that polyamines might be involved since spermine and spermidine are known to stimulate triacylglycerol synthesis in adipose tissue [5] and liver [6]. These compounds stimulate the activity of glycerol phosphate acyltransferase [5–7] and diacylglycerol acyltransferase [5] and they inhibit acyl-CoA hydrolase [5]. They are known to bind to cell membranes [6] and they interact with acyl-CoA esters. It therefore seemed likely that they might modify the translocation of phosphatidate phosphohydrolase that is induced by fatty acids and acyl-CoA esters. Here, we have shown that spermine promotes the translocation of phosphatidate phosphohydrolase from the

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soluble to the microsomal fraction when added alone, and that it facilitates the oleate-induced translocation.

2. MATERIALS AND METHODS

The sources of the rats and most of the materials have been described [4,8,9]. Spermine, spermidine and putrescine were purchased from the Sigma (London) Chemical Co. (Poole, Dorset).

The 180000 × g·min (18000 × g for 10 min) supernatant from rat liver was prepared and incubated at 37°C for 10 min with various combinations of oleate and polyamines. The supernatant

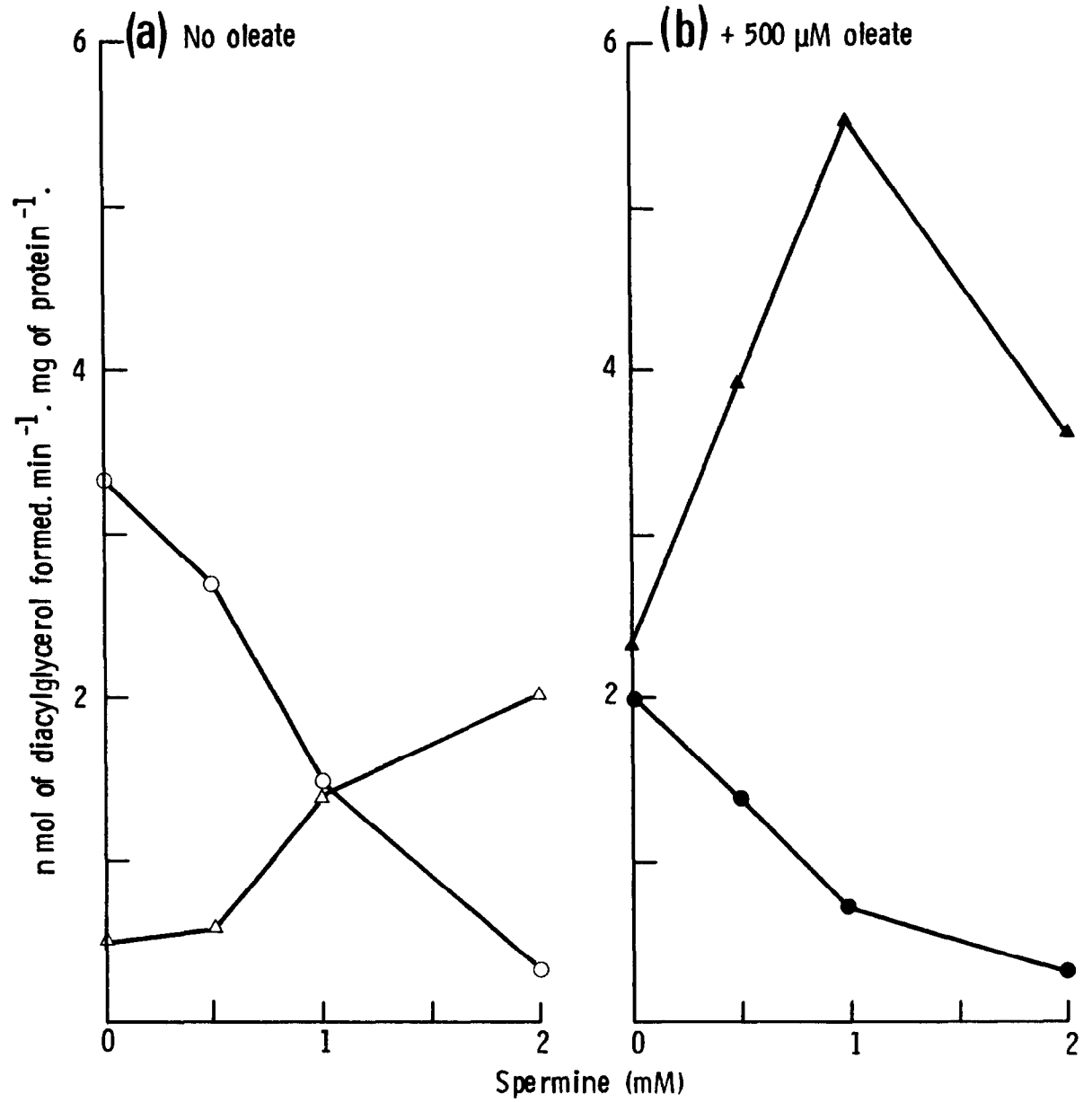

Fig.1. The effects of spermine on the specific activity of phosphatidate phosphohydrolase in the microsomal and soluble fraction of rat liver. The 180000 × g·min supernatant of rat liver was incubated for 10 min at 37°C with spermine in the absence (a) or presence (b) of 500 μM oleate, as indicated. It was then separated into a microsomal (△, ▲), and a soluble fraction (○, ●) by centrifugation (see section 2).

was then cooled to 4°C and the microsomal and soluble fractions were separated by centrifugation at 90000 × g (r_{av} = 6.3 cm) for 90 min [4]. Protein concentrations and the activity of phosphatidate phosphohydrolase were determined as in [4].

3. RESULTS AND DISCUSSION

The addition of spermine to the 180000 × g·min supernatant caused the translocation of phosphatidate phosphohydrolase from the soluble to the microsomal fraction in the presence or absence of oleate (table 1; fig.1). It also increased the action of oleate in promoting the translocation (table 1) such that lower concentrations of oleate were able to translocate the phosphohydrolase to the membranes (fig.2). Spermidine had no significant effect on the translocation when added alone, but in two independent experiments it produced an additional translocation above that obtained with oleate (table 1). Putrescine had no significant effect either alone, or in combination with oleate (table 1).

Spermine might have caused an apparent translocation by inhibiting the cytosolic phosphatidate phosphohydrolase while stimulating that in the microsomal fraction. This was checked using fractions that had been separated prior to incubation with 0.5–2.0 mM spermine. The cytosolic phosphohydrolase was inhibited by 17–39% and the microsomal activity was inhibited by 50–75%. This loss of activity is also seen in table 1 when the fractions were separated after incubation with spermine.

An alternative explanation for the apparent translocation could be that spermine promoted the aggregation of the soluble phosphohydrolase. To investigate this, the 180000 × g·min supernatant was centrifuged twice to remove more than 99% of the rotenone-insensitive cytochrome c reductase that was used as a marker for the endoplasmic reticulum [4]. When 1 mM spermine was incubated with this soluble fraction an average of 11% more of the phosphohydrolase was pelleted after centrifugation in three independent experiments. However, 32% more of the

Table 1

Effects of polyamines and oleate on the translocation of phosphatidate phosphohydrolase between the microsomal membranes and the soluble fraction of rat liver

Additions	Relative distribution of phosphatidate phosphohydrolase activity (%)		Total activity of phosphohydrolase relative to control
	Soluble fraction	Microsomal fraction	
None (control)	93 ± 3	8 ± 3	1 (13)
Spermine	67 ± 7	33 ± 7	0.68 ± 0.07 (1)
Spermidine	93 ± 0	7 ± 0	0.95 ± 0.05 (2)
Putrescine	95 ± 0.5	5 ± 0.5	0.98 ± 0 (2)
Oleate	54 ± 12	46 ± 12	0.83 ± 0.1 (9)
Oleate + spermine	23 ± 5	77 ± 5	0.67 ± 0.1 (6)
Oleate + spermidine	33 ± 5	67 ± 5	0.85 ± 0.03 (2)
Oleate + putrescine	54 ± 5	46 ± 5	0.78 ± 0.01 (2)

The 180000 × g·min supernatant was incubated for 10 min in the presence of 500 μM oleate or 1 mM polyamine as indicated, and the microsomal and soluble fractions were then separated by centrifugation (section 2). The relative phosphohydrolase activities are quoted as means ± SD where there are more than 2 independent experiments, or ± ranges for 2 independent experiments as indicated in parentheses. The mean phosphohydrolase activity of the control was 2.09 ± 0.43 nmol diacylglycerol formed·min^{-1}·mg protein^{-1}

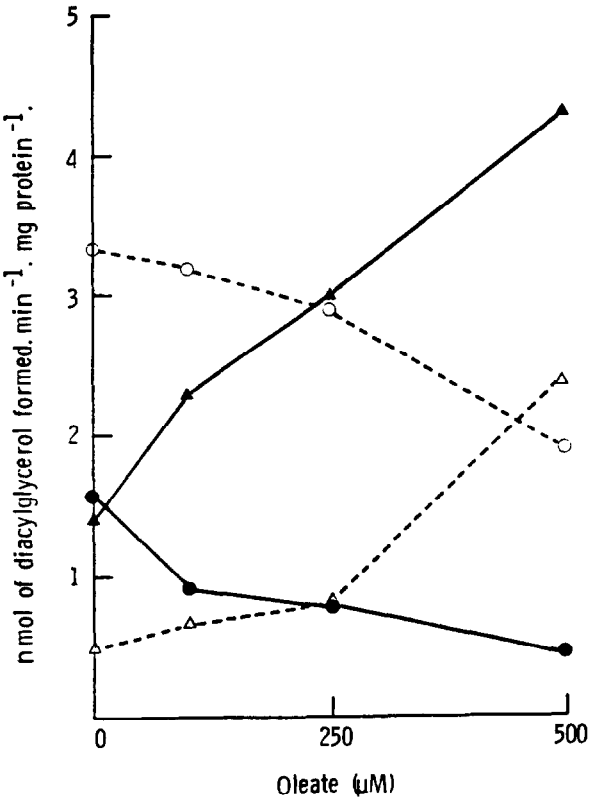

Fig.2. Interaction of spermine and oleate in promoting the translocation of phosphatidate phosphohydrolase from the soluble to the microsomal fraction of rat liver. The 180000 × *g*·min supernatant of rat liver was incubated for 10 min at 37°C with oleate in the absence (○, △) or presence (●, ▲) of 1 mM spermine. It was then separated into a microsomal (△, ▲) and a soluble (○, ●) fraction by centrifugation (see section 2).

phosphohydrolase was recovered with the pellet when the microsomal fraction was recombined with the soluble fraction. When 1 mM spermine together with 0.5 mM oleate was added to the combined soluble and microsomal fractions 63% more of the phosphohydrolase was recovered in the pellet and this was decreased to 51% more when the soluble fraction alone was treated. In order to determine the nature of this later phosphohydrolase activity from the soluble fraction, the sample was also centrifuged on a Percoll gradient. The portion of the phosphohydrolase activity that did not coincide with the soluble marker lactate dehydrogenase, sedimented with a peak that contained part of the residual rotenone-insensitive cytochrome *c* reductase and 28–40% of the added oleate (unpublished). We conclude that spermine facilitates the interaction of the phosphohydrolase with added oleate, with endogenous lipids, lipophilic proteins and with microsomal membranes.

At present we are not certain of the exact mechanism by which spermine stimulates this interaction of phosphatidate phosphohydrolase. Since it is effective in the absence of added oleate, it is likely that its interaction with the membranes of the endoplasmic reticulum facilitates the attachment of the phosphohydrolase. Furthermore, spermine is known to form complexes with acyl-CoA esters (and probably with fatty acids) and that this is probably the basis for the stimulation of glycerol phosphate acyltransferase and diacylglycerol acyltransferase activities [5–7]. In this respect it is relevant to note that the stimulation of triacylglycerol synthesis observed in [5] was greater with spermine than with spermidine and that putrescine was ineffective. The same order of potency was observed in the present experiments (table 1). Spermine does not appear to stimulate the total phosphatidate phosphohydrolase activity (table 1, [5]). However, the translocation of more of the enzyme onto the membranes together with increases in the activity of glycerol phosphate and diacylglycerol acyltransferase could explain the increased flux of precursors into triacylglycerol [5].

The physiological role of polyamines in controlling the rate of triacylglycerol synthesis is not understood, but there are several points at which regulation could occur. Polyamines can alter the effects of Ca^{2+} in controlling metabolism [10], and they might modify the stimulation of phosphatidate phosphohydrolase and triacylglycerol synthesis that is produced by vasopressin [9]. The synthesis of polyamines is thought to be regulated by ornithine decarboxylase. This enzyme is induced in the liver and in hepatoma cells by cyclic AMP and glucocorticoids and its activity rises in conditions of metabolic stress including partial hepatectomy [11,12]. In this respect the control resembles that of tyrosine aminotransferase and phosphatidate phosphohydrolase [8,13–15]. Thus the increased availability of polyamines could augment the action of fatty acids in stress conditions in translocating phosphatidate phosphohydrolase to the membranes of the endoplasmic reticulum, thus ac-

tivating the increased pool of cytosolic enzyme that is caused by the high concentrations of glucocorticoids and cyclic AMP. This would facilitate the increase in triacylglycerol synthesis which is seen after partial hepatectomy as a fatty liver [16]. Paradoxically, polyamines can also inhibit the effects of cyclic AMP and glucocorticoids in inducing the synthesis of tyrosine aminotransferase [17] and phosphatidate phosphohydrolase (unpublished). Thus polyamines could reduce the extent of the increase in the total phosphohydrolase activity while increasing the proportion of the enzyme that is metabolically active.

Further work with polyamines should be valuable to understand the complex relationship between the soluble and membrane-bound forms of phosphatidate phosphohydrolase and its interaction with other enzymes in controlling triacylglycerol synthesis.

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REFERENCES

[1] Brindley, D.N. and Sturton, R.G. (1982) New Comp. Biochem. 4, 179–213.
[2] Lamb, R.G. and McCue, S.B. (1983) Biochim. Biophys. Acta 753, 356–363.
[3] Cascales, C., Mangiapane, E.H. and Brindley, D.N. (1984) Biochem. J. 219, 911–916.
[4] Martin-Sanz, P., Hopewell, R. and Brindley, D.N. (1984) FEBS Lett. 275, 284–288.
[5] Jamdar, S.C. (1977) Arch. Biochem. Biophys. 182, 723–731.
[6] Jamdar, S.C. (1979) Arch. Biochem. Biophys. 195, 81–94.
[7] Bates, E.J. and Saggerson, E.D. (1981) Biochem. Soc. Trans. 9, 57–58.
[8] Jennings, R.J., Lawson, N., Fears, R. and Brindley, D.N. (1981) FEBS Lett. 113, 119–122.
[9] Pollard, A.D. and Brindley, D.N. (1984) Biochem. J. 217, 461–469.
[10] Theoharides, T.C. (1980) Life Sci. 27, 703–713.
[11] Canellakis, Z.N. and Theoharides, T.C. (1976) J. Biol. Chem. 251, 4436–4441.
[12] Holtta, E. (1975) Biochim. Biophys. Acta 399, 420–427.
[13] Mangiapane, E.H., Lloyd-Davies, K.A. and Brindley, D.N. (1973) Biochem. J. 134, 103–112.
[14] Lehtonen, M.A., Savolainen, M.J. and Hassinen, I.E. (1979) FEBS Lett. 99, 162–166.
[15] Pittner, R.A., Fears, R. and Brindley, D.N. (1985) Biochem. J., in press.
[16] Vance, D.E. and Pelech, S.L. (1984) Trends Biochem. Sci. 9, 17–20.
[17] Auberger, P., Samson, M. and Le Cam, A. (1983) Biochem. J. 214, 679–685.