Effects of free fatty acids on the enzymic synthesis of diacyl and ether types of choline and ethanolamine phosphoglycerides

A. Radominska-Pyrek,[1] J. Strosznajder,[2] Z. Dabrowiecki,[2] T. Chojnacki,[1] and L. A. Horrocks[3]

Polish Academy of Sciences, Warsaw, Poland; and Department of Physiological Chemistry, Ohio State University, Columbus, Ohio 43210

Abstract Activities of ethanolaminephosphotransferases (EC 2.7.8.1) and choline phosphotransferases (EC 2.7.8.2) in microsomal fractions from brains and livers of mature rats are increased several fold by the addition of 1,2-diacyl-*sn*-glycerols or 1-alkyl-2-acyl-*sn*-glycerols. Oleic acid added with diacylglycerols stimulated further the synthesis of lecithins by liver microsomes, confirming the work of Sribney and Lyman (*Can. J. Biochem.* **51:** 1479–1486, 1973). With alkylacylglycerols, oleic and stearic acids were inhibitory and linoleic acid was even more inhibitory for the synthesis of both 1-alkyl-2-acyl-*sn*-glycero-3-phosphorylcholines and the corresponding ethanolamine compounds with microsomes from both tissues. Free fatty acids without added diglycerides had mixed effects. These results are best explained by postulating the presence of two isoenzymes each for ethanolaminephosphotransferase and cholinephosphotransferase of which only one is affected by free fatty acids. Regulation of the phosphotransferases by free fatty acids may determine the proportion of CDP-choline and CDP-ethanolamine used for synthesis of diacyl and alkylacyl types of these phosphoglycerides.

Supplementary key words brain · liver · microsomes · cholinephosphotransferase (EC 2.7.8.2) · ethanolaminephosphotransferase (EC 2.7.8.1) · plasmalogen · phosphatidyl ethanolamine · phosphatidyl choline · 1-alkyl-2-acyl-*sn*-glycero-3-phosphorylcholines · 1-alkyl-2-acyl-*sn*-glycero-3-phosphorylethanolamines · stearic acid · oleic acid · linoleic acid

Synthesis of the various types of ethanolamine phosphoglycerides and choline phosphoglycerides takes place in the microsomes (1). Liver and brain contain the enzymes CDP-ethanolamine (1,2-diacyl-*sn*-glycerol ethanolaminephosphotransferase (EC 2.7.8.1)) and CDP-choline, 1,2-diacyl-*sn*-glycerol cholinephosphotransferase (EC 2.7.8.2)) that catalyze the formation of diacyl-GPE and diacyl-GPC respectively (1–3). The same enzymes probably also catalyze the formation of alkylacyl-GPE and alkylacyl-GPC from the cytidine nucleotide and 1-alkyl-2-acyl-*sn*-glycerols (4, 5). Ethanolamine plasmalogens (alkenylacyl-GPE) are formed from alkylacyl-GPE (5, 6) and the choline plasmalogens are probably formed from alkylacyl-GPC by an analogous reaction (5).

The control of the biosynthesis of the various types of ethanolamine phosphoglycerides and choline phosphoglycerides is not well understood (1). ATP inhibits the ethanolaminephosphotransferase of rat brain microsomes, particularly for the synthesis of alkylacyl-GPE (7). Sribney and Lyman (8) have recently reported that chicken liver microsomal cholinephosphotransferase is markedly stimulated by oleic acid in the presence of diacylglycerols at less than saturating concentrations. Some stimulation was also observed with other unsaturated fatty acids such as palmitoleic, arachidonic and linoleic acids. Palmitic and stearic acids had no effect on the cholinephosphotransferase and oleic acid only slightly stimulated the ethanolaminephosphotransferase. Sribney and Lyman (8) suggested that the unsaturated fatty acids cause a change in the conformation of the cholinephosphotransferase such that it is more able to accept the diacylglycerol substrate.

In the present study, we have examined the effects of free fatty acids on the cholinephosphotransferases and ethanolaminephosphotransferases of rat brain and liver with alkylacylglycerols and diacylglycerols as the diglyceride substrates.

Abbreviations: GPC, *sn*-glycero-3-phosphorylcholines; GPE, *sn*-glycero-3-phosphorylethanolamines.

[1] Institute of Biochemistry and Biophysics, Polish Academy of Sciences, ul. Rakowiecka 36, 02-532 Warsaw, Poland.

[2] Experimental and Clinical Medical Research Center, Polish Academy of Sciences, Warsaw, Poland.

[3] Department of Physiological Chemistry, The Ohio State University, Columbus, Ohio 43210.

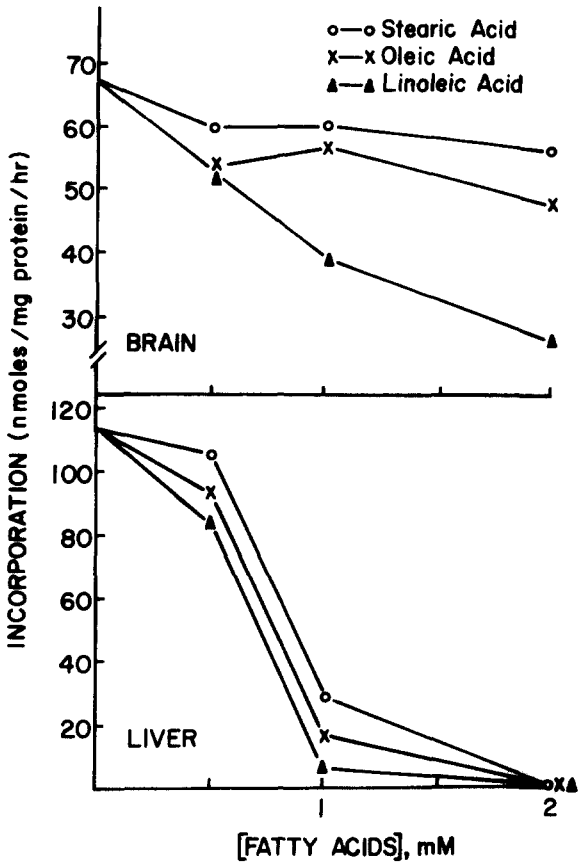

Fig. 1. Effect of increasing concentrations of fatty acids on the incorporation of radioactivity from CDP-[^{14}C]choline into lipid by microsomes from rat brain and liver. Assays were performed as described in Table 1, except that fatty acids were added as indicated and the lipid substrate was 4 mM alkylacylglycerols.

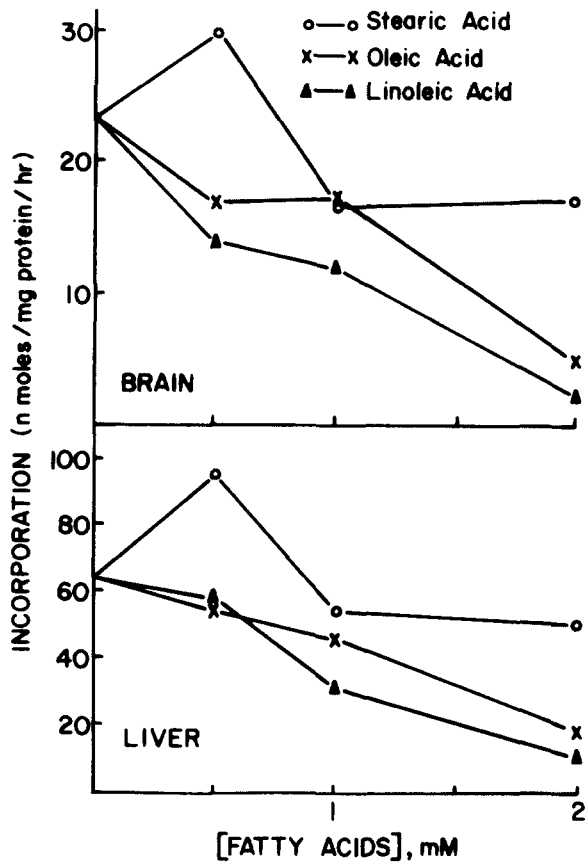

Fig. 2. Effects of increasing concentrations of fatty acids on the incorporation of radioactivity from CDP-[^{14}C]ethanolamine into lipid by microsomes from rat brain and liver. Assays were performed as described in Table 1, except that fatty acids were added as indicated, and the lipid substrate was 4 mM alkylacylglycerols.

METHODS

Materials

Microsomes from rat brains and livers were prepared as described previously (4, 5). The preparations were placed in a freezer at −20°C for one month or less. The preparation of CDP-[^{14}C]ethanolamine and the preparation of the emulsions of alkylacylglycerols and diacylglycerols were described previously (5). CDP[^{14}C]choline (60 Ci/mole) was from the Radiochemical Center, Amersham, Bucks, England.

Stearic acid was obtained from the Sigma Chemical Co., St. Louis, Mo., and oleic and linoleic acids were obtained from BDH, Laboratory Chemicals Division, Poole, England. The unsaturated fatty acids were purified (9) immediately before use. The fatty acids were suspended at a concentration of 20 mM in 0.1 M Tris-HCl, pH 7.4, which contained 0.1% Tween 20. The pH was adjusted if necessary and then the suspension was sonicated for 3–5 min for stearic acid and 1–2 min for the unsaturated fatty acids. The fatty acid preparations were transparent.

Assay of cholinephosphotransferase and ethanolaminephosphotransferase activities

Assay mixtures as indicated in the tables and figures were incubated with shaking in a water bath. Reactions were stopped after 30 min of incubation at 37°C by the addition of 3.0 ml of chloroform–methanol 2:1 (v/v). Lipids were extracted (4) and the radioactivity was assayed either with the total lipids or after separation–reaction–separation TLC (4, 5).

RESULTS

In the presence of alkylacylglycerols, the phosphotransferases were inhibited by free fatty acids (**Figs. 1** and **2**). The inhibition by free fatty acids of choline phosphoglyceride synthesis by liver microsomes was slight at 0.5 mM, almost complete at 1 mM,

and complete at 2 mM (Fig. 1). Brain microsomes were not markedly inhibited by stearic acid and oleic acid, but linoleic acid inhibited by more than 50% at 2 mM. The inhibition of the cholinephosphotransferase was always in the order linoleate > oleate > stearate. The results with ethanolaminephosphotransferases (Fig. 2) were more complex, but quite similar for brain and liver microsomes. At 0.5 mM, stearic acid activated, while oleic and linoleic acids had very little effect with liver microsomes but slightly inhibited the brain microsomes. At 1 mM, inhibitions were slight for stearic and oleic acids but about 50% for linoleic acid. At 2 mM, the inhibition by oleic and linoleic acids was almost complete. The ethanolaminephosphotransferases were always inhibited by oleic and linoleic acids. The latter was generally the stronger inhibitor.

Free fatty acid effects at 1 mM concentration were examined with alkylacylglycerols, diacylglycerols and endogenous diglycerides (**Table 1**). For choline phosphoglyceride synthesis, the free fatty acids had opposite effects in the presence of alkylacylglycerols and diacylglycerols. For example, with liver microsomes, oleic acid stimulated by 50% in the presence of diacylglycerols and inhibited by 77% in the presence of alkylacylglycerols. The stimulatory effect with diacylglycerols was quite specific for oleic acid. Linoleic acid had no effect with diacylglycerols but inhibited by 92% with alkylacylglycerols and liver microsomes. With endogenous diglycerides, a stimulation was effected by stearic acid but oleic and linoleic acids were inhibitory. For ethanolamine phosphoglyceride synthesis, no stimulation by oleic acid with diacylglycerols was found with liver microsomes. However, with brain microsomes oleic and linoleic acids were both moderately strong inhibitors. With alkylacylglycerols, all three fatty acids were inhibitory with both brain and liver microsomes. With endogenous diglycerides, synthesis of ethanolamine phosphoglycerides by brain microsomes was stimulated by stearic and oleic acid. Oleic and linoleic acids were strong inhibitors of the synthesis of ethanolamine phosphoglycerides by liver microsomes.

We then examined the effects of stearic and linoleic acids on the synthesis of each type of phosphoglyceride in conditions favoring the synthesis of ether types (Tables 2 and 3). With CDP-choline and brain microsomes (**Table 2**), stearic and linoleic acids partially inhibited the synthesis of plasmalogens. With liver microsomes, both fatty acids inhibited the synthesis of all three types of choline phosphoglycerides. Linoleic acid also inhibited the conversion of alkylacyl-GPC to alkenylacyl-GPC because only 5% of the radioactivity in ether lipids was in plasmalogens with linoleic acid, as compared with a control value of 11%. With CDP-ethanolamine and brain microsomes (**Table 3**), the inhibition by both fatty acids was greater for alkylacyl-GPE than for diacyl-GPE. Stearic acid with liver microsomes changed the proportions of the ethanolamine phosphoglyceride types synthesized.

The proportions of diacyl-GPE and alkenylacyl-GPE were decreased with a corresponding increase of alkylacyl-GPE. Linoleic acid produced some inhibi-

TABLE 1. Effect of various fatty acids on the ethanolaminephosphotransferase and cholinephosphotransferase activities of microsomes from rat brain and rat liver

Addition		Incorporation into Choline Phosphoglycerides		Incorporation into Ethanolamine Phosphoglycerides	
Diglyceride	Fatty Acid	Brain	Liver	Brain	Liver
		nmoles/mg protein/hr			
4 mM Alkylacylglycerols	None	72.8 ± 2.0[a]	94.6 ± 11.2	25.4 ± 2.2	64.3 ± 3.3
	Stearic	52.3 ± 5.2 (−28)[b]	33.3 ± 2.1 (−65)	17.8 ± 0.9 (−30)	51.8 ± 2.3 (−20)
	Oleic	64.8 ± 1.1 (−11)	22.0 ± 4.6 (−77)	18.1 ± 3.0 (−29)	46.8 ± 0.2 (−23)
	Linoleic	39.6 ± 2.8 (−46)	7.4 ± 0.2 (−92)	16.1 ± 2.1 (−37)	31.1 ± 1.2 (−52)
2 mM Diacylglycerols	None	84.3 ± 2.9	151.5 ± 6.5	34.9 ± 1.5	52.8 ± 3.3
	Stearic	74.1 ± 4.6 (−12)	167.6 ± 12.8 (+11)	25.7 ± 1.4 (−26)	42.8 ± 0.8 (−19)
	Oleic	103.5 ± 1.5 (+23)	228.1 ± 14.6 (+50)	20.0 ± 0.2 (−43)	55.9 ± 7.0 (+6)
	Linoleic	93.2 ± 4.0 (+10)	147.0 ± 8.2 (−3)	15.8 ± 0.6 (−55)	42.5 ± 7.4 (−20)
None	None	26.2 ± 1.8	24.4 ± 3.9	10.2 ± 0.5	32.2 ± 1.0
	Stearic	31.1 ± 1.3 (+19)	46.2 ± 3.9 (+89)	13.9 ± 1.4 (+36)	25.8 ± 1.3 (−20)
	Oleic	22.6 ± 1.6 (−14)	16.1 ± 1.1 (−34)	13.0 ± 0.8 (+27)	11.7 ± 1.8 (−64)
	Linoleic	18.3 ± 0.6 (−30)	13.4 ± 1.0 (−45)	10.8 ± 1.2 (+6)	5.7 ± 1.1 (−82)

Incubation mixture: 75 mM Tris-HCl, pH 7.6; 30 mM $MgCl_2$; 0.1% Tween 20; 0.63 mM CDP-[^{14}C]choline (1.27×10^5 dpm) or 0.71 mM CDP-[^{14}C]ethanolamine (1.22×10^5 dpm); 0.10 mM dithiothreitol and 0.50 mg of microsomal protein from rat brain or liver. Fatty acids (1.0 mM) were added as specified above. Incubation was for 30 min at 37°C in a final volume of 0.20 ml.

[a] The values are the mean ± SEM from three experiments.

[b] Values in parentheses are percentage stimulation.

TABLE 2. Effect of stearic and linoleic acids on the distribution of radioactivity in choline phosphoglycerides synthesized in the presence of alkylacylglycerols

Addition	Diacyl-GPC	Alkenylacyl-GPC	Alkylacyl-GPC
	nmoles/mg protein/hr		
	Brain		
None	11.4 ± 0.8[a]	4.4 ± 0.5	0.8 ± 0.1
Alkylacylglycerols	4.8 ± 0.5	7.2 ± 0.4	38.8 ± 3.3
Alkylacylglycerols, stearic acid	5.1 ± 0.2	4.9 ± 0.3	42.3 ± 1.8
Alkylacylglycerols, linoleic acid	3.9 ± 0.4	4.2 ± 0.0	39.2 ± 1.0
	Liver		
None	32.4 ± 3.8	3.3 ± 0.3	2.2 ± 0.3
Alkylacylglycerols	21.1 ± 2.3	6.6 ± 0.8	66.0 ± 4.8
Alkylacylglycerols, stearic acid	9.3 ± 1.0	3.0 ± 0.4	26.1 ± 1.9
Alkylacylglycerols, linoleic acid	4.1 ± 0.3	0.2 ± 0.0	4.0 ± 0.5

Assays were performed as described in Table 1 except that alkylacylglycerols (4 mM) and fatty acids (1 mM) were added as specified above and 0.92 mM CDP-[^{14}C]choline (4.85×10^4 dpm) was used in one experiment.

[a] Each value represents the mean ± SEM from three experiments.

tion which was more pronounced for the diacyl-GPE and the alkenylacyl-GPE than for the alkylacyl-GPE. Both fatty acids inhibited the conversion of alkylacyl-GPE to alkenylacyl-GPE by liver microsomes. Plasmalogens contained 18% of the radioactivity in ether lipids in the control, 12% with stearic acid and 10% with linoleic acid.

DISCUSSION

Effects of free fatty acids on phosphoglyceride synthesis

Oleic acid stimulated the synthesis of diacyl-GPC by liver microsomes in the presence of diacylglycerols at a less than saturating concentration, confirming the report of Sribney and Lyman (8). The degree of stimulation was somewhat less in the present study, probably because we used 2 mM instead of 0.8 mM diacylglycerols. Sribney and Lyman found substantial stimulation by linoleic acid in a chicken liver microsomal system, but we did not find an effect by this fatty acid with rat liver microsomes. Oleic acid also stimulated brain cholinephosphotransferase with diacylglycerols.

Free fatty acids were inhibitors in the presence of alkylacylglycerols. Liver cholinephosphotransferase was markedly inhibited, while liver ethanolaminephosphotransferase and both brain enzymic activities were moderately inhibited. Linoleic acid was the most effective inhibitor followed by oleic and stearic acids. Linoleic acid also inhibited the desaturation of alkylacyl-GPE and alkylacyl-GPC to the corresponding plasmalogens in the liver.

Mixed effects were observed when only endogenous diglycerides were present. Chicken liver cholinephosphotransferase was not stimulated by oleic acid in the absence of added diglyceride (8). With rat liver cholinephosphotransferase, a 34% inhibition was observed, confirming the observations of McMurray (10) and Kanoh and Ohno (11). Other effects ranged from an 82% inhibition of liver ethanolaminephosphotransferase by linoleic acid to a 36% stimulation of brain ethanolaminephosphotransferase and an 89% stimulation of liver cholinephosphotransferase by stearic acid. With only endogenous diglycerides, stearic acid was most stimulatory and linoleic acid was most inhibitory. The physiological significance of these observations is unknown since the free fatty acid concentration in normal brain is about 0.3 μmoles/g tissue (12).

Regulation of phosphotransferase

Cholinephosphotransferase and ethanolaminephosphotransferase activities may be present in the same enzyme (1). The relative lack of specificity for the nitrogen base (13, 14) and the inhibition of ethanolamine phosphotransferases by CDP-choline (15, 16), together with the inhibition of cholinephosphotransferases by CDP-ethanolamine (17), could

TABLE 3. Effect of stearic and linoleic acids on the distribution of radioactivity in ethanolamine phosphoglycerides synthesized in the presence of alkylacylglycerols

Addition	Diacyl-GPE	Alkenylacyl-GPE	Alkylacyl-GPE
	nmoles/mg protein/hr		
	Brain		
None	7.3, 6.3	1.9, 2.4	0.1, 0.1
Alkylacylglycerols	4.2, 4.7	2.4, 2.4	11.2, 12.5
Alkylacylglycerols, stearic acid	3.7, 3.8	1.9, 2.3	7.9, 6.1
Alkylacylglycerols, linoleic acid	2.4, 2.9	1.4, 1.4	6.1, 5.4
	Liver		
None	24.7, 23.3	2.2, 2.3	1.2, 0.9
Alkylacylglycerols	16.5, 17.1	5.1, 6.4	27.8, 25.8
Alkylacylglycerols, stearic acid	15.3, 11.1	3.7, 5.5	32.4, 32.6
Alkylacylglycerols, linoleic acid	9.9, 9.1	2.7, 2.0	23.2, 21.1

Assays were performed as described in Table 1 except that alkylacylglycerols (4 mM) and fatty acids (1 mM) were added as specified above. Values are given from two experiments.

be explained by a single enzyme for both activities. Kennedy and Weiss (2) suggested that the liver enzymes were different because of marked differences in stability to an increase in temperature. Differences in stability of the brain enzymes have also been noted (16, 18).

Liver enzymes respond differently to acyl CoA (19) and fatty acids (9 and the present results). The solubilization of ethanolaminephosphotransferase from rat liver microsomes (20) with very little cholinephosphotransferase activity present in the soluble extract is persuasive evidence for different enzymes for these two activities. The marked differences in fatty acid effects on these two phosphotransferase activities are additional evidence for different enzymes.

The same enzyme probably transfers phosphorylethanolamine to diacyglycerols, alkenylacylglycerols, or alkylacylglycerols (4, 15). This conclusion is based on similar K_m values for each type of diglyceride and for CDP-ethanolamine with the different diglycerides. Also, the formation of diacyl-GPE is markedly inhibited by alkylacylglycerols. Free fatty acids have similar effects on brain ethanolaminephosphotransferase in the presence of diacylglycerols or alkylacylglycerols, but their effects on liver ethanolaminephosphotransferase are quite different. Also, there are differences in the inhibition of brain ethanolaminephosphotransferase by ATP in the presence of alkylacylglycerols or diacylglycerols (7). Thus, the phosphotransferases may be branch points for the utilization of cytidine diphosphate bases depending on whether alkylacylglycerols or diacylglycerols are utilized as the other substrate.

Free fatty acids may change the conformation of the phosphotransferases, particularly liver cholinephosphotransferase, so that they are more able to accept diacylglycerols (8). Under the same conditions, the utilization of alkylacylglycerols is markedly decreased. Thus, the opposite effects of free fatty acids may be due to an effect on the same enzyme brought about by changing the conformation of the diglyceride binding site.

The effects of free fatty acids do not explain the differences between brain and liver phosphotransferases or between ethanolaminephosphotransferases and cholinephosphotransferases. These differences could be due to the presence of different proportions of an allosteric isozyme and a nonallosteric isozyme. According to this argument, the liver cholinephosphotransferase would have the highest proportion and the brain ethanolaminephosphotransferase would have the lowest proportion of the allosteric isozyme. The presence of at least two cholinephosphotransferase isozymes in brain has been suggested on the basis of different K_m values for CDP-choline for the glial and neuronal enzymes (17, 21).

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