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Phospholipid acylation by mouse sciatic nerve microsomes

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The partition of 0.3 nmol of [1-^{14}C]oleoyl-CoA in the microsomes (10 μg proteins) from mouse sciatic nerves is unaffected by the presence of lysophospholipids and is about 45% of the total oleoyl-CoA (77% of the acylglycerophosphocholine partition in the membrane). The concentration of both oleoyl-CoA and acylglycerophosphocholine is over 1 mM in the membrane. There is a selective acyl transfer from acyl-CoA to lysolipid acceptors (oleoyl > myristoyl, palmitoyl, stearoyl ≫ eicosanoyl > docosanoyl, tetracosanoyl). The exogenous acyl acceptors are acylglycerophosphocholine and acylglycerophosphoinositol and to a lesser extent acylglycerophosphoethanolamine, but not acylglycerophosphoserine. A PC formation from acylGPC in the absence of exogenous acyl donors or from oleoyl-CoA in the absence of exogenous acyl acceptor was also observed.

Introduction

The fatty acids of the peripheral nervous system of the mouse fall roughly into two categories: the ubiquitous fatty acids, chiefly saturated and unsaturated C_{18} acyl chains, are mostly associated with phospholipids, whereas the C_{20}–C_{24} fatty acids, considered as myelin markers, are specially abundant in the sphingolipids.

We have shown previously that the very long chain fatty acid content parallels exactly that of glycosphingolipids during development of the normal mouse sciatic nerves whereas the level of ubiquitous fatty acids was similar to that of phospholipids during the same period of time [1,2].

These results asked the question of the insertion of the acyl groups into the various lipids of the peripheral nervous system of the mouse. As a first contribution to this topic, we reported recently a study concerning the acyl transfer from stearoyl-CoA to a lipid acceptor, the lysophosphatidylcholine in mouse sciatic nerves [3]. As reported for acyl-CoA : acyl-GPC acyltransferase [4,10], we observed that this transacylation is strictly membrane-bound, uses only the membrane-bound acyl-CoAs, and is chiefly located in the microsomal fraction. The synthesis of labeled diacyl-GPC, is linear as a function of time (for at least 30 min) and protein amount (up to 30 μg). The activity increases as a function of the stearoyl-CoA concentration up to 100 μM (apparent K_M about 20 μM). No inhibition is observed in the whole acyl-CoA concentration range, as long as, the acyl-CoA (nmol) to protein (μg) ratio does not reach 0.5.

The acyl-CoA : acyl-GPC acyltransferase is so far the most studied transfer activity. However, even if its importance should not be underestimated and further information must be gained on its functioning simply because it may be one of the privileged ways in bringing a non-random distribution of fatty acids, other acyl-CoA acyltransferases have to be taken into account; it is the case, for example of the acyl-CoA : lysophosphatidylethanolamine, acyl-CoA : lysophosphatidylinositol, and acyl-CoA : lysophosphatidylserine acyltransferases, which have not been so far evidenced in a great variety of cell systems [4,7,8,11].

The acyl-CoA acyltransferases have not been evidenced in the peripheral nervous system; they should be investigated and compared to the potential acyl transfer from PC or acyl-GPC to acyl acceptors catalyzed by transacylase activities. These transacylase activities have been already observed chiefly in bovine

Abbreviations: 1-acyl-GPC, 1-acyl-*sn*-3-glycerophosphocholine; 1-acyl-GPE, 1-acyl-*sn*-3-glycerophosphoethanolamine; 1-acyl-GPS, 1-acyl-*sn*-3-glycerophosphoserine; 1-acyl-GPI, 1-acyl-*sn*-3-glycerophosphoinositol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; HPTLC, high performance thin-layer chromatography.

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and dog heart microsomes [12,13], but also in rabbit liver [14] and rat platelets [15]. These considerations, combined to the fact that, in vitro, the acyl-CoA : lysolipid acyltransferases require membrane-bound acyl-CoA led us to measure the binding of the substrates to the membranes and to study the different reactions allowing the acyl insertion into phospholipids.

Materials and Methods

[1-^{14}C]oleoyl-CoA (52.3 Ci/mol), 1-[1-^{14}C]palmitoylglycerophosphocholine (58.5 Ci/mol), di[1-^{14}C]palmitoylglycerophosphocholine (58 Ci/mol) and [2-^{14}C]malonyl-CoA (58.8 Ci/mol) were from Amersham, Les Ulis (France). Saturated monoacylglycerophosphocholine, oleoyl-GPC, oleoyl-GPE, saturated (C_{16}, C_{18}) monoacyl GPI, saturated (C_{16}, C_{18}) monoacyl GPS, NADPH, stearoyl-CoA, oleoyl-CoA and BSA were from Sigma Chemical Co., St Louis, MO (U.S.A.).

Preparation of membrane fractions

Membrane fractions were prepared as described in Ref. 3. Briefly mouse sciatic nerves from the B6CBA strain, were removed and homogenized in 0.05 M Tris-HCl (pH 7.5) at 4°C. The homogenate was spun at 20000 × *g* for 20 min, and the 20000 × *g* supernatant was centrifuged at 150000 × *g* for 90 min. The 150000 × *g* pellet (microsomes) was resuspended in 0.05 M. Tris-HCl (pH 7.5). The proteins were estimated by the method of Bradford [16].

Binding of the substrates

The study of substrate binding was carried out in a final volume of 50 μl in 0.05 M Tris-HCl (pH 7.5) at 37°C. Routinely reactions were initiated by the addition to the reaction mixture containing the radioactive substrates, of 10 μg microsomal proteins. The reaction mixture was centrifuged with a Beckman TL 100, at 184000 × *g* for 10 min and yielded a membrane pellet (P_1) and a supernatant (S_1). The lipid radioactivity was analyzed by HPTLC followed by radioautography (see 'Lipid analysis'). Eventually, the P_1 pellet was resuspended in 0.05 M Tris-HCl (pH 7.5) and centrifuged again at 184000 × *g* for 10 min. The radioactivity of the pellet and supernatant was measured in a Packard 2000 CA scintillation counter after addition of 2 ml of liquid scintillation cocktail (Optifluor) to 5 μl of P_1 or S_1.

Reactions of transacylation and free fatty acid formation

(1) Acyl-CoAs (saturated C_{14} to C_{24}-acyl-CoAs, oleoyl-CoA), 11 μM 1-[1-^{14}C]palmitoyl-GPC (58.5 Ci/mol) were added to 15 μg microsomal proteins in 0.05 M Tris-HCl (pH 7.5), 1 mg/ml BSA (final volume 50 μl) and incubated for 30 min. at 37°C. The incubation was stopped by the addition of 300 μl $CHCl_3/CH_3OH$ (1 : 1, v/v).

(2) 0.46 nmol [1-^{14}C] oleoyl-CoA, with or without 0.5 nmol lysophospholipid, with or without 50 μg BSA, were added to 10 μg microsomal proteins in 50 μl 0.05 M Tris-HCl (pH 7.5), and incubated for 30 min. at 37°C. The incubation was stopped by the addition of 300 μl $CHCl_3/CH_3OH$ (1 : 1, v/v).

(3) 1 nmol [1-^{14}C]dipalmitoyl-PC, or 1 nmol 1-[1-^{14}C]palmitoyl-GPC, with or without 50 μg BSA, were added to 10 μg microsomal proteins in 50 μl 0.05 M Tris-HCl (pH 7.5), and incubated for 30 min at 37°C. The incubation was stopped by the addition of 300 μl $CHCl_3/CH_3OH$ (1 : 1, v/v).

Acyl-CoA elongation. Acyl-CoA elongation as already described in Ref. 17. Briefly 3.4 nmol [2-^{14}C] malonyl-CoA (58.8 Ci/mol), 10 nmol stearoyl-CoA or oleoyl-CoA, 100 nmol NADPH, 100 μg BSA, 50 μg microsomal proteins, with or without 2 nmol 1-acylglycerophosphocholine, with or without 0.2 μmol $ATP \cdot Mg^{2+}$ were incubated for 30 min at 37°C in 100 μl 0.05 M Tris-HCl buffer (pH 7.5).

The reaction was stopped by the addition of 600 μl $CHCl_3/CH_3OH$ (1 : 1, v/v).

Lipid analysis

(1) Aliquots of the homogeneous phases obtained by addition of 300 μl $CHCl_3/CH_3OH$ were loaded on 10 × 10 cm HPTLC Kieselgel 60 plates (Merck) and eluted according to Vitiello and Zanetta [18] with methyl acetate/*n*-propanol/ chloroform/ methanol/ 0.25% aqueous potassium chloride (25 : 25 : 25 : 10 : 9, v/v) or (25 : 25 : 28 : 10 : 7, v/v) according to Heape et al. [19]. Each of the 6 lanes of the plates was 8 mm wide and separated from each other by 7 mm.

(2) Aliquots of the homogeneous phase obtained after the assay for acyl-CoA elongation were loaded on 10 × 10 cm HPTLC Kieselgel 60 plates (Merck) and eluted with /Butanol/acetic acid/water (5 : 2 : 3, v/v) according to Juguelin and Cassagne [20], in order to resolve long chain acyl-CoAs, PC, PE and glycolipids.

(3) After elution, autoradiographs were prepared (Kodak DEF 5) to visualize the labeled lipids; the different lipid bands were scraped off directly into a scintillation vial and radioactivities were determined.

Control of the purity of the labeled substrates

The purity of the labeled substrates was checked. Complete blank runs without microsomes were carried out for each experiment. [1-^{14}C]palmitoyl-GPC and [1-^{14}C]dipalmitoyl-PC were found repeatedly pure. [1-^{14}C]oleoyl-CoA was slightly hydrolyzed and label traces were detected in the lipid bands after HPTLC. The contamination was taken into account for the calculations, and the results corrected accordingly.

Results

Binding of acylation substrates to the microsomes of mouse sciatic nerves

We have shown previously [3] that the acyl-CoAs, substrates of the acyl-CoA:lysolipid acyltransferases, are bound to the microsomes prior to their metabolism. The binding of the acyl-CoA was studied in the presence or in the absence of BSA (Juguelin et al. [30]. We further analyzed whether the binding of 18:1-CoA was modified by the amount of acyl acceptor present in the incubation mixture. Using standard conditions (0.3 nmol labeled 18:1-CoA; 0.5 nmol lysolipid; 10 μg microsomal proteins; incubation 30 min at 37°C), we measured the partition of the acyl-CoA between the membrane and the aqueous phase and we determined the label distribution in the membrane pellet and the aqueous phase. Whatever the experimental conditions the oleoyl-CoA is partly metabolized within the membrane. In the presence of 50 μg BSA, the maximal metabolism is about 12% of administered, free fatty acids are evidenced exclusively in the aqueous phase and a phospholipid formation is observed within the membrane. Because of the 12% oleoyl-CoA metabolism the determination of the radioactivity in the membranes and in the supernatant is not a strict determination but an estimation of the partition of the acyl-CoAs. The membrane-bound label accounted for 44.3 ± 2.3%, 45.6 ± 1.5 and 46.3 ± 2.5 of the total initial acyl-CoA label in the presence of 1-acyl-GPC, acyl-GPS and 1-acyl-GPE, respectively, as compared to 45 ± 1.8% in the absence of lysolipid. Thus the addition of lysolipid does not modify the binding of the acyl donor to the microsomes. The binding of 1-acyl-GPC to the membrane was also investigated (Fig. 1). There is a linear partition of the radioactivity up to 2 nmol 1-[1-^{14}C]palmitoyl-GPC, 77 ± 5.9% of the label from the acyl-GPC are bound to the membrane in the concentration range tested. 2.3 ± 0.2% of the total radioactivity were found in PC, 7.6 ± 4.2% in the free fatty acids, and 86.3 ± 2.8% in 1-acyl-GPC. Taken together these results show that 1-acyl-GPC is accumulated in the membrane. The membrane volume was estimated according to Fato et al. [29], assuming that (i) the phospholipids are dissolving the substrate, (ii) there are equal amounts of proteins and lipids in the membrane and (iii) the vesicles have a density close to 1 g/ml. It is easy to calculate that from 1 μM 1-acyl-GPC added to membranes (10 μg microsomal proteins) the intramembrane concentration may exceed 2 mM.The membranes concentrate the acyl acceptors by about 2 or 3 orders of magnitude so that the kinetic parameters should be calculated from the actual (membrane) concentration and not from the bulk aqueous concentration. The release of the labeled 1-acyl-GPC from the membrane was further investigated as a function of the membrane-bound

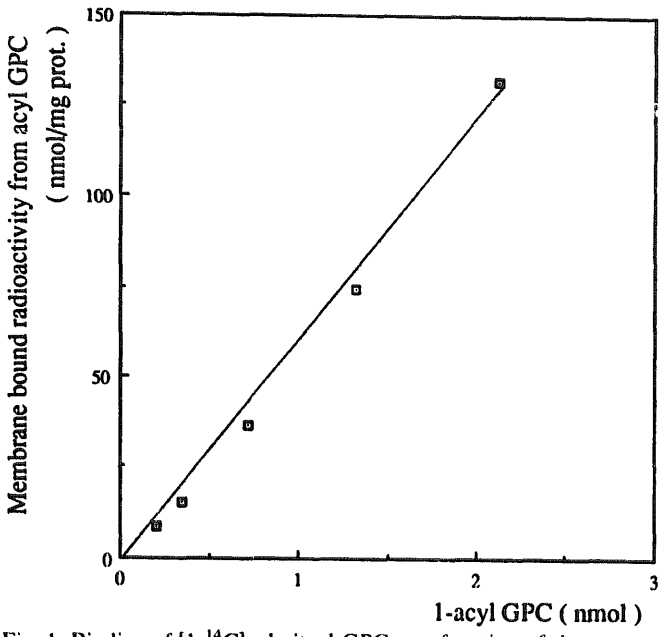

Fig. 1. Binding of [1-^{14}C]palmitoyl-GPC as a function of the amount of [1-^{14}C]palmitoyl-GPC. Various amounts of labeled palmitoyl-GPC were incubated for 30 min. at 37°C with 10 μg microsomal proteins in a final volume of 50 μl 0.05 M Tris-HCl buffer (pH 7.5). The membrane bound radioactivity was measured as described in Materials and Methods. The binding is given as the radioactivity found in the microsomes (nmol/mg protein).

1-acyl-GPC. Microsomes preloaded with various amounts of 1-acyl-GPC were resuspended and, after 2 min, fractionated by centrifugation as membrane pellet (P_2) and aqueous phase (S_2 supernatant). The label distribution of the P_2 membrane lipids was highly similar to that observed in P_1: 2.3% of the label were found in PC and 10% in the free fatty acids. More than 85% of the total radioactivity of the P_2 membrane pellet were found in 1-acyl-GPC. Since the label distribution in the membrane is identical to that of the whole reaction mixture, the distribution of the radioactivity between the membrane (P_2) and the supernatant (S_2) is an index of the lipid release and particularly of the 1-acyl-GPC release. The equilibrium is reached when 76.3 ± 4.5% of the label from 1-acyl-GPC are bound to the membrane and 23.7 ± 4.5% are released in the aqueous phase, whatever the initial amount in the microsomes (Fig. 2).

Acylation of the 1-acyl-GPC by the acyl moieties from acyl-CoAs

The reaction of acyl insertion into the membrane-bound acyl-GPC was studied as a function of the acyl chain length of the various acyl-CoAs. The results are given in Table I. C_{14}-CoA to C_{18}-CoA are substrates of the acyl-CoA:acyl-GPC acyltransferase; the specific activity is very similar for these three acyl donors. The replacement of C_{18}-CoA by 18:1-CoA leads to a marked increase of the specific activity from 3.9 to 7.7 nmol/mg proteins per 30 min. Accordingly, we further

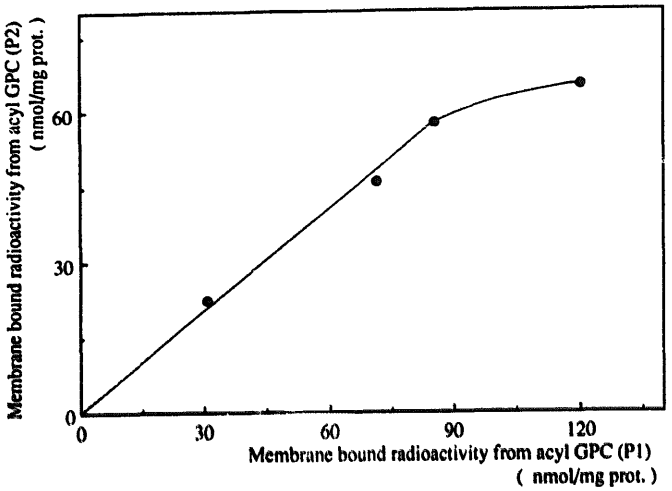

Fig. 2. Acyl-GPC release from the preloaded microsomes as a function of the membrane-bound acyl-GPC. Variable amounts of [1-^{14}C]palmitoyl-GPC (58.5 Ci/mol) were incubated with 10 μg microsomal proteins for 30 min at 37°C in 50 μl 0.05 Tris-HCl (pH 7.5). The P_1 pellet was prepared, homogenized and its radioactivity was measured. The P_2 membrane pellet and the S_2 supernatant were prepared. The results are given as the radioactivity of P_2 (nmol/mg microsomal proteins).

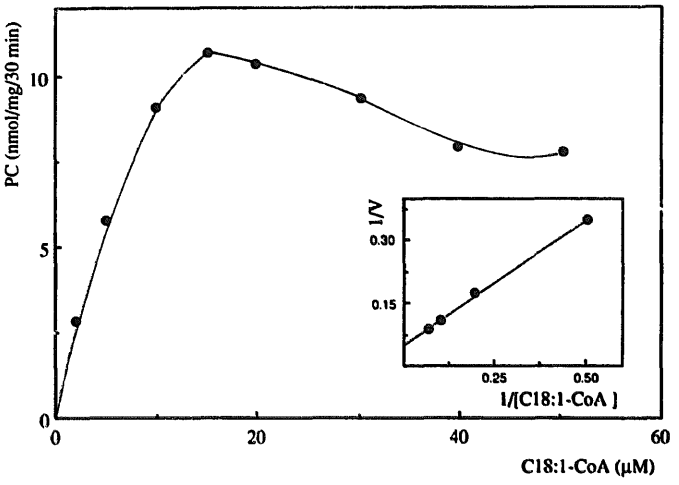

Fig. 3. Acyl-CoA:acyl-GPC acyl transferase activity as a function of the oleoyl-CoA concentration. 11 μM [1-^{14}C]palmitoyl-GPC (58.5 Ci/mol), 2–50 μM oleoyl-CoA were incubated for 30 min with 15 μg microsomal proteins at 37°C in 50 μl 0.05 M-Tris HCl (pH 7.5) and BSA (50 μg). Results are given as the nmol PC synthesized/mg protein/30 min (mean value of two experiments).

investigated the acyl-CoA:acyl-GPC acyltransferase as a function of the oleoyl-CoA concentration (Fig. 3). The insertion of the oleoyl moiety into 1-acyl-GPC increased as a function of the substrate concentration up to 15 μM (total) oleoyl-CoA (about 36 nmol bound oleoyl-CoA/mg microsomal protein); it reached a maximum and decreased slightly up to 50 μM total oleoyl-CoA (about 115 nmol bound oleoyl-CoA/mg microsomal protein). The maximal activity was 10.5 nmol/mg protein per 30 min. at 15 μM 18:1-CoA as compared to 7.7 at 50 μM oleoyl-CoA. At the maximal activity about 30% of the acyl moieties from the acyl-CoAs bound to the membranes have been transferred to acyl-GPC. Under the conditions used in this experiment (0.55 nmol 1-acyl-GPC, 15 μg protein) it may be calculated from Fig. 1 that there are about 30 nmol 1-acyl-GPC bound/mg of membrane proteins, so that about 35% of the acyl acceptor are acylated in 30 min. This estimation is in excellent agreement with those made from Table I. The inhibition observed above 15 μM oleoyl-CoA is not due to a solubilization because as high as 150 nmol oleoyl-CoA/mg microsomal protein may be used without any noticeable modification of the membrane recovery [3]. Using the first part of the curve, we estimated the kinetic parameters of the oleoyl-CoA:1-acyl-GPC transacylase (Fig. 3, inset); we found $V_{Max} = 20$ nmol/mg protein per 30 min, and $K_{M,app} = 12.5$ μM. This latter value may also be evaluated from the 18:1-CoA bound to the membrane. In this case, the $K_{M,app}$ may be 29.6 nmol/mg protein. These estimations reflect the concentration parameter due to the partition of most of the substrate in the small volume occupied by the membrane vesicles.

TABLE I

PC Formation catalyzed by mouse sciatic nerve microsomes

Activities were measured at 11 μM [1-^{14}C]palmitoyl-GPC (58.5 Ci/mol) and when used, 50 μM acyl-CoAs, using 0.015 mg microsomal proteins and 50 μg BSA incubated for 30 min. at 37°C, in 50 Kl 0.05 Tris-HCl (pH 7.5). The activity refers to the incorporation of radioactivity into PC. Mean value ± S.D. of separate experiments. Or mean value of two experiments (C_{22}-CoA, C_{24}-CoA).

Substrate	Activity (nmol/mg protein per 30 min)
None	0.3
C_{14}-CoA	5.4 ± 1.5
C_{16}-CoA	4.9 ± 0.8
C_{18}-CoA	3.9 ± 0.2
18:1-CoA	7.7 ± 0.8
C_{20}-CoA	0.9 ± 0.2
C_{22}-CoA	0.2
C_{24}-CoA	0.3

From Table I it is also remarkable that the C_{20}–C_{24} acyl-CoAs are apparently not acyl donors for the acyl-CoA:acyl-GPC acyltransferases, since the acyl transfer from C_{20}-CoA, reaches only 20% of the activity with C_{18}-CoA, whereas the C_{22}-CoA and C_{24}-CoA are still poorer acyl donors (6 and 8% of the reaction with C_{18}-CoA, respectively). This marked difference is in very good agreement with the well-known distribution of the very long chain fatty acids which are nearly absent from PC. This question was further investigated by studying the eventual insertion of labeled very long acyl chains from acyl-CoAs synthesized endogenously by the acyl-CoA elongase located within the microsomes of mouse sciatic nerves. The elongation of unlabeled stearoyl-CoA was carried.out by the addition of malonyl-CoA and NADPH. The results are shown in

TABLE II

Acyl-CoA elongase and acyl-CoA transferase activities in mouse sciatic nerves microsomes

Acyl-CoA elongase activity was measured at 34 μM [2-^{14}C]malonyl-CoA (58.8 Ci/mol), 100 μM stearoyl-CoA or oleoyl-CoA, 1 mM NADPH, 100 μg BSA, 50 μg microsomal proteins, with or without 2 mM ATP·Mg^{2+}. The activity refers to the incorporation of radioactivity from malonyl-CoA into very long chain acyl-CoAs. Acyl-CoA acyltransferase activity refers to the incorporation of radioactivity from C_{18}-CoA or 18:1-CoA into PC (lines 1 and 2); in the experimental conditions of Table I, the activity represents the incorporation of radioactivity from [1-^{14}C]palmitoyl-GPC into PC (lines 3 and 4); under experimental conditions allowing C_{18}-CoA or 18:1-CoA elongation, the acyl-CoA acyltransferase activity represent the incorporation of radioactivity from labeled very long chain acyl-CoA into PC (lines 5–11).

Substrate	Acyl-CoA elongase (nmol/mg protein per 30 min)	Acyl-CoA acyltransferase (nmol/mg protein per 30 min)
[1-^{14}C]C_{18}-CoA	0	0
[1-^{14}C]18:1-CoA	0	3
C_{18}-CoA + [1-^{14}C]palmitoyl-GPC	–	4
18:1-CoA + [1-^{14}C]palmitoyl-GPC	–	7, 7
C_{18}-CoA + [2-^{14}C]malonyl-CoA + 1-acyl-GPC	3.6	0
C_{18}-CoA + [2-^{14}C]malonyl-CoA + 1-acyl-GPC + ATP·Mg^{2+}	3.2	0
18:1-CoA + [2-^{14}C]malonyl-CoA + 1-acyl-GPC (30 min)	3.6	0
18:1-CoA + [2-^{14}C]malonyl-CoA + 1-acyl-GPC (120 min)	2.5	0
18:1-CoA + [2-^{14}C]malonyl-CoA + 1-acyl-GPE (120 min)	1.3	0
18:1-CoA + [2-^{14}C]malonyl-CoA + 1-acyl-GPS (120 min)	1.7	0

Table II. The HPTLC analysis of the whole reaction mixture allows to visualize not only the long chain acyl-CoAs but also PC, PS, PE, cerebrosides and free fatty acids [20]. As expected from a previous study [17], there is a high synthesis of radioactive very long chain acyl-CoAs from unlabeled stearoyl-CoA and labeled malonyl-CoA (about 24 nmol/mg protein per 30 min). No label, however is observed in any phospholipid. The addition of 1-acyl-GPC to the reaction mixture results in a marked decrease of the elongation rate (about 3.6 nmol/mg protein per 30 min), but no label is observed in PC in the absence or in the presence of ATP-Mg. This lack of PC formation is not due to a decrease of the activity of the acyl-CoA : acyl-GPC acyltransferase since the replacement of unlabeled by labeled acyl-GPC allows to evidence a normal PC synthesis. This experiment shows that stearoyl CoA is accepted by the acyl-CoA : acyl-GPC acyltransferases but not the neosynthesized saturated C_{20}–C_{24} acyl-CoAs. On the other hand, in total agreement with a previous observation [3], there is no acyl transfer from stearoyl-CoA to PC in the absence of 1-acyl-GPC (Table II, line 1).

A similar analysis may be drawn from the experiments done in the presence of oleoyl-CoA instead of stearoyl-CoA. Oleoyl-CoA may be elongated by labeled malonyl-CoA (Table II) and the products of the elongase are released as acyl-CoAs. Oleoyl-CoA is also the substrate of an acyltransferase able to form PC in the absence or in the presence of 1-acyl-GPC (Table II). In the absence of exogenous acyl acceptor, depending on experimental conditions, 5–7% of the oleoyl-CoA may be inserted into phospholipids. However, under conditions allowing the formation of radioactive very long chain acyl-CoAs from unlabeled oleoyl-CoA, no label is detected in PC upon addition of 1-acyl-GPC, whatever the time of incubation with 1-acyl-GPC (30 min or 120 min). The replacement of 1-acyl-GPC by 1-acyl-GPE or 1-acyl-GPS decreases the overall unlabeled oleoyl-CoA elongation with labeled malonyl-CoA but no labeled PE or PS formation is observed.

Acylation of endogenous acyl acceptors

The transfer of the oleoyl moiety of 18:1-CoA to lipids in the absence of exogenous acyl acceptors has been further investigated. To 10 μg microsomal proteins were added 0.46 nmol [1-^{14}C]oleoyl-CoA for 30 min at 37°C. The analysis of the label distribution among the lipids is given in Table III. As expected from a previous work [3,21], there is a high level of acyl-CoA hydrolysis (about 20%) which is considerably lowered upon addition of 50 μg BSA. Besides this predicted hydrolysis of the substrate, there is a net transfer of acyl moieties to chiefly PC and PE (3.1 and 2.2% of administered, respectively). Traces of labeled PS and no PI are detected. The addition of BSA, though reducing the rate of substrate hydrolysis does not greatly modify the acylation of endogenous acceptors. An analysis of the microsomal lipids according to

TABLE III

Acylation of endogenous acceptors

Phospholipid acylation was measured at 9.2 μM [1-^{14}C]oleoyl-CoA (52.3 Ci/mol) (lines 1 and 2) or 20 μM [1-^{14}C]dipalmitoyl-PC (58 Ci/mol) (line 3), using 10 μg microsomal proteins, with or without 50 μg BSA, incubated for 30 min at 37°C in 50 μl 0.05 M Tris-HCl (pH 7.5). The activity refers to the % incorporation of the radioactivity from the acyl donor to the acyl acceptor(s). Two experiments done in duplicate (lines 1,2); four experiments ± S.D. (line 3). n.d., not detected.

Substrate	Acylation (% of administered)				
	PC	PS	PI	PE	FFA
[1-^{14}C]Oleoyl-CoA	3.1	0.3	n.d.	2.2	19.8
[1-^{14}C]Oleoyl-CoA + BSA	2.4	0.9	n.d.	1.7	6.7
[1-^{14}C]Dipalmitoyl-PC		1.8 ±0.1	1 ±0.1	0.9 ±0.4	3.6 ±1.7

Heape et al. [19] did not reveal the presence of measurable amounts of endogenous lysolipids. The direct assay of lipids is sensitive enough to allow the conclusion that no endogenous lysophospholipid acceptors are present as long as the acyl moieties of the lysophospholipids are unsaturated. On the other hand, endogenous acyl GPC may be detected by the formation of labeled PC from stearoyl-CoA in the absence of exogenous acyl-GPC. This method allows to detect as little as 0.1 nmol lysophospholipid/mg protein [3]. The fact that there is no PC formation from labeled stearoyl-CoA suggests that, if present, preformed endogenous acyl-GPC is less than 0.1 nmol/mg protein. Replacing stearoyl-CoA by oleoyl-CoA allows the formation of labeled phospholipids: approx. 5% of the administered label are found in phospholipids (2.2 nmol oleate moieties inserted into lipids/mg protein) and approx. 3% in PC (1.3 nmol/mg protein). These latter values are far higher than the 0.1 nmol preformed acyl-GPC/mg protein that the method is able to detect. An interpretation of these results is that the oleoyl-CoA acyltransferases use unknown endogenous acyl acceptors in addition to the exogenous acyl-GPC. When 0.5 nmol labeled acyl-GPC is added to 10 μg microsomes and incubated for 30 min at 37°C, it is partly hydrolyzed (about 10%); 2% of the label are recovered in PC and to a lesser extent in PS (0.7%). No label is associated with PE.

Using labeled PC instead of 1-acyl GPC (1 nmol; 12 μg microsomal proteins) we determined that 93.7 $\pm$ 2.8% were associated with the membrane, and that there is a spontaneous release of PC which may account for about 15% of the membrane-bound phosphatidylcholine. After a 30 min incubation at 37°C, we observed the formation of acyl-GPC (2 $\pm$ 0.5% of administered label) and 3.6 $\pm$ 1.7% of the radioactivity were found in the free fatty acids. Label was also detected in PI and PE, and the radioactivity associated with PS reached 1.8 $\pm$ 0.15% of administered (Table III). The addition of BSA did not provoke a marked modification of the label of acyl-GPC (which decreased slightly); PS, PI and PE were unchanged. A weak hydrolysis of PC was also observed. The total acyl transfer from PC to the other phospholipids reached 5.8 $\pm$ 0.9% of the administered label.

Acylation of exogenous acceptors

Experiments were carried out in order to analyze the insertion of the oleoyl moiety from labeled oleoyl-CoA into three potentially important acyl acceptors, acyl-GPE, acyl-GPI and acyl-GPS, in addition to that already shown in acyl-GPC. To the microsomes (10 μg) were added [1-^{14}C]oleoyl-CoA (0.5 nmol), and exogenous acyl acceptors (0.5 nmol). After a 30 min incubation the lipids were analyzed by HPTLC. In each case autoradiographies were prepared and the radioactivity of each lipid band was measured. The results are given in Table IV. As the acylation of endogenous (unknown) acceptors is present in any case, the acylation of exogenous acceptors is defined as the difference of the label found in any lipid in the presence ('specific acylation') or in the absence (unknown acceptor) of external lysolipid. With 1-acyl-GPC the acylation is specific for PC, with the exception of a faint labeling of PS which accounts for 1.5% of the administered label. The addition of BSA does not modify the acylation pattern. The use of acyl-GPE instead of acyl-GPC leads to a specific increase in PE labeling which may reach 50% of that observed in PC from acyl-GPC. Some label was detected in PC, PS but not in PI, in the presence or in the absence of BSA. In the presence of acyl-GPI, there is a high label insertion into PI and to a far lesser extent into PC; the acyl-CoA : acyl-GPI acyltransferase has the same level of activity as the acyl-CoA : acyl-GPC acyltransferase. As observed with the other lysophospholipids, the addition of BSA does not modify greatly the activity (which is slightly lowered as with 1-acyl-GPC).

TABLE IV

Acyl transfer to various lysophospholipids from [1-^{14}C]oleoyl-CoA

Acyl transfer was measured at 9.2 μM [1-^{14}C]oleoyl-CoA (52.3 Ci/mol) and 10 μM lysophospholipid, with or without 50 μg BSA, using 10 μg microsomal proteins, incubated for 30 min at 37°C in 50 μl 0.05 M Tris-HCl (pH 7.5). The activity refers to the specific incorporation of radioactivity (% of administered into the phospholipids, calculated as the difference of the radioactivity observed in the presence and in the absence of lysophospholipid; results are mean value of two experiments done in duplicate. n.d., not detected. FFA, free fatty acids.

Lysophospholipid	Product (% of administered label)						
	acyl-GPC	PC	PS	PI	PE	FFA	Total acylation
Acyl-GPC	0	9.9	1.4	n.d.	0	0	11.3
Acyl-GPC + BSA	1.4	7.8	1.5	n.d.	0.5	1.3	11.2
Acyl-GPE	1.3	1	0.7	n.d.	5.3	2.1	8.3
Acyl-GPE + BSA	0	2.2	1.8	n.d.	4.2	3.4	8.2
Acyl-GPI	0	2.2	0.1	9.1	0.3	0.7	11.7
Acyl-GPI + BSA	0	3.3	0.1	7.8	1.0	4.8	12.2
Acyl-GPS	0	0	0	0	0	3.7	0

Discussion

The study of the mechanisms of the acyl transfer to the various membrane lipids (chiefly phospholipids) has recently gained more attention. It has been shown that the fatty acids are largely incorporated into phospholipids via the acyl-CoA : lysophospholipid acyltransferases. This acylation system has been extensively studied, chiefly the acyl-CoA : 1-acylglycerophosphocholine acyltransferase [4–10], and in some cases extended to other acyl acceptors. The activity may depend on the nature of the acyl moiety to be transferred, and of the acyl acceptor. In vitro, the kinetics of the reaction depend also on the availability of the substrates in the vicinity of the enzymes, which is regulated by the binding of the substrates to the membrane [22,23]. Under the standard experimental conditions used throughout this study, the 1-acyl-GPC, when used, is largely concentrated in the membrane (almost 80% of administered) with the consequence of a dramatic increase in its actual concentration. 45% of administered oleoyl-CoA are also located in the microsomes, and this partition is not modified upon lysophospholipid addition. Though there is a very high concentration of these substrates in the microsomes, no noticeable solubilization of the membrane was detected for example by an increased protein release in the supernatant fraction, in good agreement with our previous observations [3]. The clear-cut specificity of the acyl-CoA : 1-acyl-GPC acyltransferase towards the CoA thiol esters of the saturated and unsaturated 18 carbon fatty acids, the nearly absence of transfer from the saturated C_{20}- to C_{24}-acyl-CoA to 1-acyl-GPC, allow to interpret why very long chain fatty acids even though synthesized in the microsomes from mouse sciatic nerves [17] are not accumulated in phosphatidylcholine [24]. To our knowledge no other study of the saturated C_{20}–C_{24} acyl-CoA transfer to lyso-PC has ever been reported. In the case of polyunsaturated fatty acids it has been shown repeatedly that either unsaturated C_{18}-CoA or polyunsaturated 20 carbon fatty acyl-CoAs were the best substrates of 1-acyl-GPC acyltransferases, followed by saturated C_{16} and C_{18} acyl-CoAs. No transacylation was observed from polyunsaturated C_{22} acyl-CoAs (see, for example, Ref. 5). The activity we have measured in the presence of oleoyl-CoA and 1-acyl-GPC is similar to that observed in rat brain microsomes [7] and in both cases, the inhibition of the activity with an overall oleoyl-CoA concentration higher than 20 μM is almost identical. This inhibition was not observed when using stearoyl-CoA instead of oleoyl-CoA [3].

Besides the acyl-CoA : 1-acyl-GPC acyltransferase, other acyl-CoA : lysophospholipid transferase activities are also evidenced. These activities may play an important role in the deacylation-reacylation pathway, which regulates the composition of phospholipids and the concentration of lysophospholipids and free fatty acids [25]. In heart muscle microsomes, 1-acyl-GPI shows a greater preference than 1-acyl-GPC for arachidonoyl-CoA, a fact which may explain the preponderance of this acyl group in PI [11]. The activities reported in this study are high relatively to oleoyl-CoA : 1-acyl-GPC acyltransferase, particularly in the case of 1-acyl-GPI as acceptor, and higher than that reported in rat brain microsomes [7].

It is not known whether this reaction, as well as that involving 1-acyl-GPE is catalyzed by the acyl-CoA : 1-acyl-GPC transacylase. The total absence of PS formation from oleoyl-CoA and 1-acyl-GPS could be due to the absence of an acyl-CoA : 1-acyl-GPS acyltransferase activity, or, if the activity exists, the enzyme and the substrate could be located on distinct leaflets of the membrane. To test this possibility, Triton X-100 (0.01%) was added to the reaction mixture; no formation of labeled PS was observed. In the case of the microsomes from mouse sciatic nerves, the action of a deacylation-reacylation pathway is not likely to insure the distinct fatty acid composition of this lipid, as demonstrated in other tissues [26].

Recently new acylation systems have been evidenced in various mammalian cells; the enzymes involved catalyze the acyl insertion into a lysolipid from a diacyl phospholipid in the absence of CoA and ATP [27,28]. This type of reaction may be stimulated upon CoA addition [13,27]; an increased activity may be obtained upon CoA + ATP-Mg addition [8,13,15,27].

It is highly likely that in the mammalian cells the fatty acid composition of the phospholipids is regulated by diverse and complex mechanisms involving various types of transferases and/or transacylases.

The complexity of the mechanisms involved is illustrated by the results obtained by adding labeled PC to the microsomes. Reddy and Schmid observed a weak acylation of PS, PI and PE in the absence of exogenous lysophospholipid [13]. The PS labeling reached 0.03% of administered label; 0.05% were found in PI and 0.14% in PE. In our hands, the values reached as high as 2% in PS, and approx. 1% in PI and in PE; moreover, these results do not take into account the potential dilution of the labeled PC with endogenous microsomal PC. The addition of 50 μM 1-acyl-GPS or 1-acyl-GPE to the reaction mixture did not change the level of transacylation from PC, in marked contrast to the situation observed in dog heart microsomes [13], since the addition of 50 μM 1-acyl-GPS or 1-acyl-GPE led in this case to a large stimulation of label transfer (which reached 5.5% to 9% of administered labeled in PS and about 2% in PE).

In rat brain microsomes [8] almost no transacylation was observed from labeled PC to PE in the absence of 1-acyl-GPE and it reached 0.5–1% of the PC label in

the presence of 50 μM of 1-palmitoyl-GPE. The high transacylation observed in the case of mouse sciatic nerve microsomes in the absence of exogenous acceptors is correlated to the formation of acyl-GPC (2.0 ± 0.5% of administered). The free fatty acid formation was not diminished by the addition of BSA. This suggests that a phospholipase could be coupled to the PC transacylase during the transacylation process. The formation of radioactive PC from [1-^{14}C]acyl-GPC could be a mere acylation or transacylation with endogenous acyldonors or could be due to a 1-acyl-GPC: 1-acyl-GPC transacylase activity. This activity, where evidenced is specific for 1-acyl-GPC, and, when purified is not accompanied by the appearance of labeled free fatty acids [12]. In the mouse sciatic nerves microsomes, there is also a partial hydrolysis of 1-acyl-GPC indicating the occurence of a lysophospholipase. The PC formation from 1-acyl-GPC will be further investigated and the eventual occurence of endogenous acyldonors will be investigated.

The reaction involving the oleoyl transfer from oleoyl-CoA to various lipids (chiefly PC and acyl-GPC) in the absence of exogenous acceptors is to our knowledge, shown for the first time, and its mechanism is unknown. It exhibits a marked preference for unsaturated acyl-CoAs since no transfer occurs from stearoyl CoA (this study and Ref. 3). This activity and the concomitant formation of acyl-GPC, will be further analyzed and particular attention will be paid to the determination (or formation during the reaction) of the acyl endogenous acceptors.

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