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PHOSPHOLIPID METABOLISM OF STIMULATED LYMPHOCYTES

PREFERENTIAL INCORPORATION OF POLYUNSATURATED FATTY ACIDS INTO PLASMA MEMBRANE PHOSPHOLIPID UPON STIMULATION WITH CONCANAVALIN A

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Rabbit thymocytes were isolated and incubated for various lengths of time with concanavalin A. The cultures were pulsed for the last 12.5 min of incubation with equimolar mixtures of radioactively labelled fatty acids, either [^{3}H]arachidonate and [^{14}C]oleate or [^{3}H]arachidonate and [^{14}C]palmitate, and the uptake of each fatty acid into phospholipid of plasma membrane was determined. Upon binding of the mitogen, the fatty acids were incorporated at an increased rate with a new steady state being reached between 12.5 and 42.5 min after stimulation. Initially after 12.5 min, when the two fatty acids were added together, no preferential incorporation of the polyunsaturated fatty acid arachidonate was seen compared to the saturated or monounsaturated ones, palmitate or oleate. However shortly thereafter arachidonate, when compared to palmitate or oleate, started to be preferentially incorporated into plasma membrane phospholipid so that by 4 h after activation, only arachidonate was incorporated at an increased rate: the uptake of palmitate and oleate had reverted to that of unstimulated cells. In contrast, when palmitate or oleate were added alone, after 4 h of activation incorporation was increased similar to that of arachidonate, suggesting that all long chain fatty acids compete for the same activated enyzme(s). A detailed analysis of incorporation into phospholipid species showed that all fatty acids were taken up with the highest rate into phosphatidylcholine. After activation, fatty acid incorporation was increased by approx. 50% for phosphatidylcholine: the highest stimulation rates were observed with phosphatidylinositol (3–7-fold) and phosphatidylethanolamine (2–3-fold). The data suggest that shortly after stimulation with mitogens, the membrane phospholipids start to change by replacing saturated and monounsaturated fatty acids by polyunsaturated ones, thus creating a new membrane.

Introduction

Among the earliest changes occurring after lymphocyte stimulation is the elevated incorporation of long chain fatty acids into the phospholipids of the plasma membrane [1,2], not because of an enhanced de novo synthesis of the phospholipids, but rather due to an increased turnover of the fatty acid moieties [3,4]. This turnover appears to originate in an enhanced deacylation of the membrane phospholipids by phospholipase A [4] and subsequent reacylation with acyl-CoA:lysophosphoacylglycerol *O*-acyltransferases [5,6].

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Abbreviation: Hepes, 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid.

After activation with mitogens it has been found that the ratio of polyenoic to saturated fatty acids is increased in the phospholipids of the cells [7]. Since acyl-CoA:lysophosphatidylcholine *O*-acyltransferase has a higher affinity for the polyunsaturated fatty acids [5,6] than for the more saturated ones, it is believed that the increase in the level of polyunsaturated fatty acids found after activation is due to the preferential incorporation of these fatty acids by the acyltransferases.

In this communication, the results of testing this hypothesis directly will be presented, i.e. if there is preferential incorporation of polyunsaturated fatty acids into phospholipid upon activation. At various times after activation with concanavalin A, thymocytes were pulsed with two differently radioactively labelled fatty acids of varying saturation and the incorporation into plasma membrane phospholipid determined. It will be shown that the polyunsaturated long chain fatty acid arachidonate was incorporated into phospholipids at a relatively higher rate than the saturated fatty acid palmitate and the monounsaturated fatty acid oleate.

Materials and Methods

Cells and cell culture. The thymi from rabbits were removed immediately after exsanguination and placed in phosphate-buffered saline. A cell suspension was made by macerating pieces of thymus tissue in a loosely fitting tissue grinder and then filtering through a small column of nylon wool (Leukopak, Fenwal Laboratories, Morton Grove, IL, U.S.A.) to remove debris and dead cells. The cells were collected by centrifugation, washed in phosphate buffered saline and resuspended at a concentration of $5.5 \cdot 10^7$ cells/ml of Dulbecco's minimal essential medium containing 100 μg/ml streptomycin, 100 I.U./ml penicillin and 20 mmol/l Hepes, pH 7.2–7.4. All the preceding operations were carried out at room temperature. After equilibrating the cells for approximately half an hour at 37°C, concanavalin A (Pharmacia Ltd., Freiburg, F.R.G.) was added to give a final concentration of 10 μg/ml and the cells further incubated for the desired length of time. At the times indicated, an equimolar mixture of 10-fold concentrated [^{3}H]arachidonate (20:4) and [^{14}C]oleate (18:1) or [^{3}H]arachidonate and [^{14}C]palmitate (16:0) was added to give a final total concentration of 10 μmol/l (5 μmol/l each) and the cells incubated for a further 12.5 min. In some experiments [^{14}C]palmitate, [^{14}C]oleate or [^{3}H]arachidonate were added separately, each at a concentration of 10 μmol/l and the cells also incubated for a further 12.5 min. The specific activity of [^{3}H]arachidonate was 10 μCi/mmol and of [^{14}C]palmitate and [^{14}C]oleate was 4 μCi/mmol, made by mixing the labelled fatty acid with the unlabelled fatty acid. Unlabelled palmitate and oleate were obtained from Sigma Chemicals, München, F.R.G., and arachidonate from Nu Chek-Prep., Elysion, MN, U.S.A. All labelled fatty acids were obtained from Amersham Buchler, Brunswick, F.R.G. Oleate and palmitate were stored dissolved in chloroform/methanol (2:1, v/v), arachidonate in benzene/ethanol (1:4, v/v). Before an experiment, each fatty acid was separately evaporated to dryness under nitrogen and sonicated in Dulbecco's modified Eagle's medium three times for 20 s at 50 watts, which resulted in a homogeneous dispersion.

Isolation of subcellular organelles. At the end of the incubation period, the cells were put on ice and immediately collected by centrifugation, resuspended at $7.5 \cdot 10^7$ cells/ml of 0.14 mol/l KCl, 0.01 mol/l Hepes, 0.5 mmol/l $MgCl_2$, pH 7.0 and disrupted by nitrogen cavitation as described [8]. Briefly, the cells were equilibrated at 4°C to 30 atm N_2 for 20 min with gentle stirring in an Artisan pressure homogenizer (Artisan Metal Products, Watham, MA, U.S.A.) and then released dropwise after which EDTA was added to give a final concentration of 1.0 mmol/l. The nuclei were collected by centrifuging at $1200 \times g$ for 15 min in an IEC PRJ centrifuge; the large granule fraction (containing mitochondria, lysosomes, and aggregated microsomal membranes) was pelleted at $18000 \times g$ for 20 min in a Beckman J21 centrifuge and the microsomes were sedimented at $175000 \times g$ for 60 min in a Beckman Spinco L5 centrifuge, Rotor 60 Ti. The fractions were resuspended in 0.14 mol/l KCl, 0.01 mol/l Hepes, pH 7.0. The characterization of these fractions has been previously described [8]. More than 80% of the microsomal membranes prepared from thymocytes are derived from the plasma membrane [8–10], as

assessed by chemical analyses and marker enzymes, although these preparations referred to as plasma membranes were not hypotonically shocked and thus also contained trapped cytosol.

Analytical procedures. The protein content of the various fractions was measured by native fluorescence as described [11] using a Perkin-Elmer MPF-44 fluorescence spectrophotometer (Perkin-Elmer, Friedrichshafen, F.R.G.).

The lipids of the various cell organelles were extracted with chloroform/methanol as previously described [4], and then separated by thin-layer chromatography on 0.2 mm silica gel plastic sheets (TLC plastic sheets silica gel 60, Merck, Darmstadt, F.R.G.) using chloroform/methanol/water (65:40:9.5, v/v) for routine experiments and chloroform/methanol/acetic acid/0.9% NaCl (50:25:8:4, v/v) in experiments where the fraction containing phosphatidylinositol and phosphatidylserine was separated from phosphatidylcholine. In some experiments, phosphatidylinositol and phosphatidylserine were also separated by two-dimensional chromatography using chloroform/methanol/concentrated NH_3/water (65:25:5:5, v/v) as the solvent in the second direction after careful drying of the plates. Incorporation was either measured in total phospholipids (sphingomyelin, phosphatidylcholine, phosphatidylserine, phosphatidylinositol, and phosphatidylethanolamine) or into individual phospholipid fractions as indicated. Individual spots were visualized by exposure to iodine vapour, the areas cut out with scissors and counted in a Mark III Searle Nuclear Chicago liquid scintillation counter with $^3H/^{14}C$ double labelling setting. Spillover of ^{14}C into the ^3H channel was determined in control experiments and the data corrected accordingly. Spillover of ^3H into the ^{14}C channel was negligible.

Data are expressed as nmol incorporated per mg of protein. Recovery of protein/10^8 cells is shown in Table I. The range of duplicates varied less than 10% from the mean in over 90% of the duplications. For each time point, at least three different preparations of cells were used with up to six replicate determinations. Statistical analyses were carried out using the paired-sample *t*-test.

Results

Incorporation of fatty acids into phospholipids of cell organelles

Thymocytes incubated with concanavalin A for the indicated lengths of time were pulsed for the final 12.5 min of incubation with equimolar concentrations of [^{3}H]arachidonate and [^{14}C]oleate or [^{3}H]arachidonate and [^{14}C]palmitate. A final total concentration of 10 μmol/l long chain fatty acids, as used, proved to be nontoxic to lymphocytes in protein-free medium as assessed by trypan blue exclusion.

The recovery of protein in the various cell organelles of normal and concanavalin A-activated thymocytes is shown in Table I. With normal thymocytes, 50–75% of the total protein was found in the cytosol fraction, 15–25% in the nuclear fraction and the remainder divided equally between the mitochondria and plasma membrane, i.e. about 10–15% in each fraction. In contrast to our previously reported results [8], the yield of plasma membrane is comparatively high; however, the present preparations were not hypotonically shocked and thus contain trapped cytosol. With concanavalin A-activated thymocytes, there was always a decrease in the amount of protein found in the nuclear fraction with a concomitant increase in mainly the mitochondrial fraction, although a small increase was usually found in the plasma membrane and cytosol fraction as well. The meaning of this decrease in nuclear protein is unclear; it may indicate an increased fragility of the nuclei as an early consequence of binding of concanavalin A.

The incorporation of [^{3}H]arachidonate and [^{14}C]oleate into phospholipid of the various cell organelles is shown in two experiments in Table I. Less than 25% of the total fatty acid added was incorporated into phospholipid; the remainder was recovered mostly as free fatty acid associated with the cells. Upon activation with concanavalin A, the amount of fatty acid incorporated into total phospholipid per mg protein recovered from the nuclear, mitochondrial and cytosol fractions was not significantly changed or was somewhat inhibited. This is in contrast to the increased fatty

TABLE I

DISTRIBUTION OF PROTEIN AND SPECIFIC INCORPORATION OF FATTY ACID INTO PHOSPHOLIPID IN CELL ORGANELLES OF NORMAL AND CONCANAVALIN A-ACTIVATED THYMOCYTES

Thymocytes were cultured at 37°C either alone or with 10 μg/ml concanavalin A (con A) for 75 min in experiment 'a' and 240 min in experiment 'b', at which time equimolar concentrations of arachidonate and oleate were added to give a final total concentration of 10 μmol/l and incubated for a further 12.5 min. The values given are means of duplicate determinations. The plasma membranes were not shocked osmotically, and thus contain trapped cytoplasmic protein. The percentage recovery is presented as mean ± range.

	Expt.	Protein recovered ($\mu g/1.0 \cdot 10^8$ cells)		Fatty acid incorporation into phospholipid (nmol/mg of protein)			
		Controls	Con A	Arachidonate		Oleate	
				Controls	Con A	Controls	Con A
Homogenate	a	600	635	1.94	1.64	0.76	0.67
	b	455	465	1.92	1.81	0.60	0.55
Nuclei	a	120	60	3.61	3.19	1.78	2.00
	b	105	40	6.86	6.49	2.37	2.26
Large granules	a	60	95	3.54	3.49	1.49	1.74
	b	55	85	8.70	6.83	2.73	1.88
Cytosol	a	380	425	0.12	0.13	0.07	0.08
	b	280	295	0.18	0.23	0.07	0.07
Plasma membrane	a	65	80	3.24	5.10	2.35	2.82
	b	55	65	2.30	4.96	1.59	1.83
% recovery		106.5 ± 2.5	104 ± 0.0	104 ± 2.0	98 ± 5.0	100 ± 2.0	93 ± 3.0

acid incorporation into phospholipid of the plasma membranes. Similar results were found when the cells were pulsed with [^{3}H]arachidonate and [^{14}C]palmitate (data not shown). Thus the remainder of the results report only on those changes found in phospholipid metabolism in the plasma membrane.

Incorporation of fatty acids into phospholipids of the plasma membrane

The changes with time of fatty acid incorporation into total phospholipid when both fatty acids arachidonate and oleate or arachidonate and palmitate were added together at equimolar concentration is shown in Tables II and III. In the unstimulated control lymphocytes, each individual fatty acid was incorporated into plasma membrane phospholipid at approximately the same rate during the entire culture period, although after some time in culture, arachidonate was incorporated at a somewhat higher rate, probably due to in vitro adaptation [12]. When the thymocytes were activated with concanavalin A, incorporation of total fatty acids into plasma membrane phospholipid, i.e. the sum of the incorporation of the pair of fatty acids added, became measureably increased within the first 12.5 min and approached a new steady state somewhat later. The increased incorporation found immediately after addition of concanavalin A for each individual fatty acid was more pronounced (although not statistically significant) for the saturated fatty acid palmitate and the monounsaturated fatty acid oleate than for the polyunsaturated fatty acid arachidonate. However, with time, the uptake of arachidonate into phospholipid continued to increase for over the 4 hours, in contrast to the incorporation of palmitate and oleate, which slowly decreased towards that seen in unstimulated cells.

Incorporation of fatty acids into individual phospholipid species

A more detailed analysis of the incorporation of fatty acid into the individual phospholipid species was carried out on thymocytes activated for 4 h with concanavalin A. Both the incorporation of

TABLE II

SPECIFIC INCORPORATION OF FATTY ACIDS INTO PLASMA MEMBRANE PHOSPHOLIPID OF NORMAL OR CONCANAVALIN A-ACTIVATED THYMOCYTES

Thymocytes were incubated at 37°C alone or with 10 μg/ml concanavalin A; for the last 12.5 min of incubation time, equimolar concentrations of arachidonate and oleate were added to give a final concentration of 10 μmol/l. The values given are means ± S.E. of six determinations.

Length of time of concanavalin A stimulation (min)	nmol incorporated per mg protein			
	Arachidonate		Oleate	
	Controls	Concanavalin A	Controls	Concanavalin A
12.5	1.09 ± 0.22	1.70 ± 0.23 [a]	1.38 ± 0.48	2.08 ± 0.52 [b]
87.5	1.62 ± 0.38	3.10 ± 0.56 [b]	1.38 ± 0.25	1.90 ± 0.29 [a]
252.5	1.92 ± 0.35	3.99 ± 0.69 [a]	1.16 ± 0.29	1.47 ± 0.25

[a] $P < 0.01$, for concanavalin A values compared to controls.
[b] $P < 0.0005$, for concanavalin A values compared to controls.

individually added fatty acids, either palmitate, oleate, or arachidonate at a concentration of 10 μmol/l (Table IV), as well as the mixture of fatty acids (palmitate and arachidonate shown in Table V) was determined.

In unstimulated thymocytes, the majority of the fatty acid was incorporated into phosphatidylcholine. This was found for all fatty acids, i.e. palmitate, oleate, linoleate (not shown), or arachidonate when added individually or as a mixture, although the amount incorporated decreased somewhat as the degree of saturation increased. Between 7.5 and 15% of the fatty acids was incorporated into phosphatidylethanolamine and slightly less into a fraction containing phosphatidylserine and phosphatidylinositol. In both these instances, the incorporation rate increased with the degree of unsaturation with the greatest differences found (10–20-fold for arachidonate compared to palmitate) in the latter fraction.

TABLE III

SPECIFIC INCORPORATION OF FATTY ACIDS INTO PLASMA MEMBRANE PHOSPHOLIPID OF NORMAL OR CONCANAVALIN A-ACTIVATED THYMOCYTES

Thymocytes were incubated at 37°C alone or with 10 μg/ml concanavalin A; for the last 12.5 minutes of incubation time, equimolar concentrations of arachidonate and palmitate were added to give a final concentration of 10 μmol/l. The values given are means ± S.E. of six determinations.

Length of time of concanavalin A stimulation (min)	nmol incorporated per mg protein			
	Arachidonate		Palmitate	
	Controls	Concanavalin A	Controls	Concanavalin A
12.5	1.77 ± 0.21	2.72 ± 0.26 [b]	0.79 ± 0.17	1.30 ± 0.32 [a]
42.5	1.98 ± 0.35	3.55 ± 0.40 [b]	0.48 ± 0.16	0.78 ± 0.28
87.5	1.94 ± 0.15	3.48 ± 0.40 [a]	0.74 ± 0.24	0.85 ± 0.33
252.5	1.88 ± 0.15	4.14 ± 0.38 [b]	0.31 ± 0.03	0.35 ± 0.03

[a] $P < 0.005$, for concanavalin A values compared to controls.
[b] $P < 0.0005$, for concanavalin values compared to controls.

TABLE IV

SPECIFIC INCORPORATION OF FATTY ACIDS INTO PLASMA MEMBRANE PHOSPHOLIPID SPECIES OF NORMAL OR CONCANAVALIN A-ACTIVATED THYMOCYTES

Thymocytes were incubated for 4 h with or without concanavalin A. For the last 12.5 min of incubation, individual fatty acids were added to give a final concentration of 10 μmol/l. Values given are means ± range of duplicate determinations. LPC, lysophosphatidylcholine; Sphi, sphingomyelin; PC, phosphatidylcholine; PI, phosphatidylinositol; PS, phosphatidylserine; PE, phosphatidylethanolamine.

Phospholipid	nmol fatty acid incorporated per mg protein					
	Palmitate		Oleate		Arachidonate	
	Controls	Concanavalin A	Controls	Concanavalin A	Controls	Concanavalin A
LPC	0.008 ± 0.000	0.011 ± 0.003	0.044 ± 0.001	0.037 ± 0.000	0.025 ± 0.000	0.030 ± 0.000
Sphi	0.008 ± 0.003	0.021 ± 0.006	0.022 ± 0.003	0.022 ± 0.001	0.027 ± 0.005	0.039 ± 0.002
PC	0.544 ± 0.039	0.926 ± 0.024	0.436 ± 0.016	0.744 ± 0.105	0.703 ± 0.005	0.921 ± 0.034
PI/PS	0.014 ± 0.000	0.055 ± 0.008	0.063 ± 0.000	0.344 ± 0.002	0.132 ± 0.002	0.722 ± 0.032
PE	0.064 ± 0.006	0.176 ± 0.003	0.104 ± 0.022	0.269 ± 0.017	0.142 ± 0.007	0.234 ± 0.016
Total	0.638 ± 0.048	1.189 ± 0.042	0.669 ± 0.042	1.416 ± 0.125	1.029 ± 0.019	1.946 ± 0.093

In stimulated lymphocytes, the incorporation of any added fatty acid into total phospholipid increased approximately 2-fold (Table IV); however, when the mixture of fatty acids was added, increased uptake was only found with arachidonate with little change in the incorporation rate of the monounsaturated or saturated fatty acids. As shown in Table V and in agreement with the results presented in Table III, palmitate uptake into total phospholipid of activated thymocytes increased by less than 10% when added in a mixture with arachidonate in contrast to the over 1.8-fold higher incorporation when palmitate was added alone. Similarly, increased incorporation of oleate occurred upon stimulation when this fatty acid was added alone (Table IV), but was absent when

TABLE V

SPECIFIC INCORPORATION OF FATTY ACIDS INTO PLASMA MEMBRANE PHOSPHOLIPID SPECIES OF NORMAL OR CONCANAVALIN A-ACTIVATED THYMOCYTES

Thymocytes were incubated for 4 h with or without concanavalin A. For the last 12.5 min of incubation, an equimolar mixture of palmitate and arachidonate was added to give a final concentration of 10 μmol/l. Values given are means ± S.E. of four determinations. For abbreviations see Table IV.

Phospholipid	nmol fatty acid incorporated per mg protein			
	Palmitate		Arachidonate	
	Controls	Concanavalin A	Controls	Concanavalin A
LPC	0.011 ± 0.001	0.010 ± 0.002	0.038 ± 0.002	0.053 ± 0.003
Sphi	0.013 ± 0.004	0.012 ± 0.001	0.031 ± 0.005	0.054 ± 0.009
PC	0.316 ± 0.016	0.323 ± 0.021	1.510 ± 0.024	2.244 ± 0.179
PI/PS	0.011 ± 0.001	0.019 ± 0.001	0.267 ± 0.027	1.699 ± 0.183
PE	0.027 ± 0.006	0.050 ± 0.009	0.282 ± 0.010	0.619 ± 0.028
Total	0.367 ± 0.026	0.402 ± 0.003	2.088 ± 0.050	4.611 ± 0.365

oleate was added together with arachidonate (Table II). This indicates that the cells cultured for 4 h are capable of incorporating palmitate and oleate at an increased rate upon activation, but have a preference for arachidonate when present.

The increased incorporation found upon activation varied among the individual phospholipid species. Whereas incorporation into the major phospholipid phosphatidylcholine was increased by about 50% (varying from 30 to 100%), there was a 3–6-fold increased incorporation into the fraction containing phosphatidylserine and phosphatidylinositol and 2–3-fold increased incorporation into phosphatidylethanolamine. When the fraction containing phosphatidylserine and phosphatidylinositol was separated by two dimensional thin layer chromatography, it became apparent that incorporation, as well as the increased incorporation rate after stimulation were due nearly exclusively to fatty acid uptake into phosphatidylinositol (Table VI).

Incorporation of different fatty acids into plasma membrane phosphatidylcholine involves one acyl-CoA:lysophosphatidylcholine O-acyltransferase

Due to the fact that lysophosphoacylglycerol *O*-acyltransferases are integral membrane constituents, it is not known if one enzyme with different affinities for each acyl-CoA or several different enzymes each specific for an acyl-CoA is responsible for the reacylation of the lysophosphoacylglycerol. The results described above, i.e. an increase in the total enzyme activity after stimulation, accompanied by a relative increase in the incorporation of the polyenoic fatty acid arachidonate and a complementary decrease in the incorporation of saturated or monounsaturated fatty acids, could be most easily reconciled by the specific activation of one enzyme. To further test this, microsomes were incubated under optimal conditions with lysophosphatidylcholine (1-acyl-*sn*-glycero-3-phosphocholine) and radioactively labelled oleoyl-CoA in the presence of unlabelled Arachidonoyl-CoA. Because acyl-CoA:lysophosphatidylcholine *O*-acyltransferase has higher affinity for arachidonoyl-CoA than for oleoyl-CoA or palmitoyl-CoA [5], arachidonoyl-CoA should inhibit the formation of labelled phosphatidylcholine, if the acyltransferase is indeed one enzyme. As shown in Table VII, arachidonoyl-CoA effectively interfered with the incorporation of the

TABLE VI

SPECIFIC INCORPORATION OF FATTY ACIDS INTO THE PLASMA MEMBRANE PHOSPHOLIPIDS PHOSPHATIDYLINOSITOL OR PHOSPHATIDYLSERINE

Thymocytes were incubated for 4 h with or without concanavalin A. For the last 12.5 min of incubation, the fatty acids, palmitate or arachidonate, were added individually to give a final concentration of 10 μmol/l. Phosphatidylinositol (PI) and phosphatidylserine (PS) were separated by two-dimensional thin-layer chromatography as described in Materials and Methods.

	nmol fatty acid incorporation per mg protein			
	Palmitate		Arachidonate	
	Control	Concanavalin A	Control	Concanavalin A
PS	0.012	0.031	0.080	0.101
PI	0.028	0.184	0.226	0.686

TABLE VII

INHIBITION OF INCORPORATION OF OLEOYL-CoA INTO PHOSPHATIDYLCHOLINE BY ARACHIDONOYL-CoA

Acyl-CoA:lysophosphatidylcholine *O*-acyltransferase activity of rabbit thymocyte plasma membrane was determined as described [3]. Oleoyl-CoA:lysophosphatidylcholine *O*-acyltransferase was measured with [1-^{14}C]oleoyl-CoA and unlabelled 1-acyl-*sn*-glycero-3-phosphocholine in the presence of increasing concentrations of arachidonoyl-CoA. As control, enzyme activity was measured using 1-[1-^{14}C]palmitoyl-*sn*-glycero-3-phosphocholine and the indicated concentrations of arachidonoyl-CoA.

Concentration of arachidonoyl-CoA (μmol/l)	Specific activity (nmol/mg protein per min of acyl-CoA:lysophosphatidylcholine *O*-acyltransferase	
	+oleoyl-CoA	+arachidonoyl-CoA
0	2.34	-
1	1.48	1.19
2.5	0.90	2.80
5	0.50	4.80
10	0.40	8.41
30	0.34	8.74

labelled oleoyl-CoA into phosphatidylcholine at concentration of arachidonoyl-CoA which was not inhibitory for the activity towards arachidonoyl-CoA. This is further evidence that one enyzme may be responsible for the transfer of fatty acids of different degrees of saturation into phospholipids of lymphocyte plasma membrane.

Discussion

These results show that immediately after receptor ligand binding and continuing for at least several hours, there was an increased incorporation of fatty acid moieties into phospholipids of the plasma membrane without a concomitant increased incorporation into other membranes. The uptake of total fatty acid increased immediately upon activation with concanavalin A, reaching a new steady state shortly after 12.5 min with an incorporation rate approx. 2-fold higher than in the control cells. This is fairly consistent with our earlier findings [1] in that the uptake of the long chain fatty acid [^{14}C]oleate into plasma membrane phosphatidylcholine of rabbit lymph nodes was enhanced after stimulation with phytohemagglutinin (a mitogen from *Phaseolus vulgaris*), with maximal activation of acyltransferase as measured by increased uptake of fatty acid by approx. 45 min after addition of the mitogen [10]. This is in contrast to the findings of Dobson and Mellors [14], who reported inhibition of acyltransferase activity in whole cells after 4 h of concanavalin A stimulation. As may be deduced from Table I, this may result from a different behaviour of the various cell organelles during activation, including an inhibition of fatty acid incorporation into nuclei. Thus we also do not always find an increase of fatty acid uptake into whole cell homogenates but occasionally detect suppression. However, in all species tested so far, including human, pig, rabbits, rat, mouse, and chicken, we found an increase in fatty acid turnover in phospholipids of the plasma membrane upon activation of cells and accordingly, activation of the plasma membrane acyltransferase.

Immediately after activation, i.e. by 12.5 min after the addition of mitogen, there was a similar increase in incorporation of the polyenoic acid arachidonate, as of the monounsaturated or saturated ones, oleate and palmitate. Thereafter, the incorporation rate of arachidonate increased, plateauing by 40–80 minutes, while that of palmitate and oleate slowly decreased towards that seen in non-activated cells. These results are unlikely to be due to dilution effects. The amount of fatty acid added to the cells is quite large accounting for approx. 25% of the total fatty acid present in the cells [13]. Arachidonate (or linoleate which is rapidly converted to arachidonate) is present in the free fatty acid pool in higher amounts than palmitate or oleate [7], thus arachidonate should be diluted more in vivo which would favour the greater incorporation of the more saturated fatty acids. In addition, the preferential incorporation of arachidonate into phospholipid did not occur immediately, rather there was a change from higher uptake of all fatty acids to a slowly increasing preferential incorporation of arachidonate into phospholipid with time after addition of mitogen. This preferential incorporation at the expense of the more saturated ones was seen with all individual phospholipid species (Table V).

The preferential incorporation of arachidonate is not due to the inability of the more saturated fatty acids to be incorporated into plasma membrane phospholipid after culture. Palmitate and oleate, if administered individually have an increased uptake similar to arachidonate 4 h after activation. On the other hand because of the higher affinity of the acyltransferase for the polyenoic acids, their selective incorporation would seem be ensured: acyl-CoA:lysophosphatidylcholine *O*-acyltransferase which is probably one enzyme and is responsible for reacylating the main phospholipid phosphatidylcholine, has been shown to have the highest affinity for the polyunsaturated fatty acids [5,6]. Moreover, upon stimulation with concanavalin A, the activity of this enzyme was increased to a much greater extent for arachidonoyl-CoA compared to oleoyl-CoA [10].

Because arachidonic acid is incorporated at a relatively faster rate into the plasma membrane phospholipids of activated cells than the more saturated fatty acids, the ratio of polyenoic to saturated or monounsaturated fatty acids would slowly increase. We have recently shown that incorporation of polyunsaturated fatty acids into isolated lymphocyte plasma membranes, dose de-

pendently modulates the activity of several plasma membrane associated enzymes [15]: the enzyme changes observed showed a striking similarity to those found in the membranes of concanavalin A-activated lymphocytes [16]. Thus it is intriguing to speculate that the increase in the degree of phospholipid unsaturation underlies the many metabolic events pushing and maintaining the activated state in stimulated lymphocytes.

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