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LOCALIZATION OF LYSOPHOSPHATIDYLCHOLINE IN BOVINE CHROMAFFIN GRANULES

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Summary

One of the unique features of the chromaffin granule membrane is the presence of about 17 mol% lysophosphatidylcholine. Lysophosphatidylcholine isolated from the granules could be degraded by approx. 94% by lysophospholipase. This result is consistent with chemical analyses data showing that about 9% of this lysophospholipid is 1′-alkenyl glycerophosphocholine.

The localization of the acylglycerophosphocholine in the chromaffin granule membrane was studied by using pure bovine liver lysophospholipases. In intact granules only about 10% of the total lysophosphatidylcholine was directly available for enzymic hydrolysis. In contrast, when granule membranes (ghosts) were treated with lysophospholipases approx. 60% of the lysophosphatidylcholine was deacylated. These values did not increase after pre-treatment of intact granules or ghosts with trypsin. Added 1-[1-^{14}C]palmitoyl-*sn*-glycero-3-phosphocholine did not mix with the endogenous lysophosphatidylcholine pool(s) and remained completely accessible to added lysophospholipases.

Introduction

Catecholamines are stored in the adrenal medulla in specialized subcellular structures, the chromaffin granules. Upon cholinergic stimulation of the cell these granules release their content into the blood stream through an exocytosis process which is thought to involve fusion of the granule membrane with the plasma membrane [1—4]. Exocytosis, the molecular mechanism of which is not fully understood, also occurs in a number of other cells when hormones, neurotransmitters or other products are released from secretory granules into the extracellular space.

For this reason much work has been done on the (bovine) chromaffin granules (for recent reviews, see Refs. 5—7). One of the striking features of these investigations concerns the high content of lysophosphatidylcholine in granule membranes, which amounted to as much as 17% of the total lipid phosphorus of the membrane [8]. The early suggestion that lysophosphatidylcholine could play a role in the physiological fusion of membranes during exocytosis [8] has received further support when other workers demonstrated its ability to behave, at least in vitro, as a fusogenic agent [9—11]. However, a direct involvement of lysophosphatidylcholine in exocytotic processes has never been demonstrated (see Refs. 52—55 for discussion).

There is not much information available on the localization of lysophosphatidylcholine in the chromaffin granule membrane. Recently, Voyta et al. [12] have incubated purified granules with [1-^{14}C]oleoyl CoA and rat liver microsomes containing acyl-CoA : 1-acyl-*sn*-glycero-3-phosphocholine *O*-acyltransferase (EC 2.3.1.23) to determine the accessibility of the granule membrane lysophosphatidylcholine to this membrane-bound enzyme. A marked increase in accessibility was observed when broken granules were used.

Previous work from this laboratory [13—15] has demonstrated that purified bovine liver lysophospholipases (EC 3.1.1.5) are appropriate tools to investigate transmembrane distributions and movements of lysophosphatidylcholine in model membranes. In the present study we make use of this approach to get insight in the lysophosphatidylcholine distribution and movement in the transverse plane of the chromaffin granule membrane.

Materials and Methods

Materials

Ficoll 400 was obtained from Pharmacia (Uppsala, Sweden). Deuterium oxide (min. 99.75% ^2H_2O, for NMR spectroscopy), silica gel H and 60HR were products of Merck (Darmstadt, F.R.G.). 1-[1-^{14}C]Palmitoyl-*sn*-glycero-3-phosphocholine was purchased from NEN (Boston, U.S.A.). Bovine trypsin and hen egg-white trypsin-inhibitor were from Boehringer (Mannheim, F.R.G.). Lysophospholipases I and II (EC 3.1.1.5) were purified from bovine liver as described earlier [16]. Their specific activities were 1200 and 800 nmol/min per mg protein, respectively.

Methods

Preparation of chromaffin granules. Bovine adrenal glands were collected at the local slaughterhouse approx. 20 min after the animals had been killed and immediately placed on crushed ice. After rapid dissection the medullae were used to prepare purified chromaffin granules essentially as described by Trifaró and Dworkind [17]. Other published methods for granule purification [18,19] are known to yield either heavily contaminated [17] or rather fragile [20,21] granules. The isolation medium contained 0.25 M sucrose, 2 mM EDTA and 10 mM potassium phosphate buffer (pH 7.0). The solution used to form the density gradient was made up in ^2H_2O and contained 0.27 M sucrose, 19.5% Ficoll, 2 mM EDTA, 5.2 mM KH_2PO_4 and 4.8 mM K_2HPO_4. The granules were resuspended and washed twice with the isolation medium to remove the Ficoll.

The final pellet was resuspended in 0.5 M sucrose, 2 mM EDTA and 10 mM potassium phosphate buffer (pH 7.0). Unless stated otherwise, all incubations were performed in this medium (incubation buffer).

Phospholipid analysis and lysophosphatidylcholine isolation. Suspensions of freshly isolated chromaffin granules were extracted according to Folch et al. [22]. Phospholipid composition was determined after two-dimensional thin-layer chromatography [15]. Lysophosphatidylcholine was isolated on silica 60 HR thin-layer plates. These were developed with $CHCl_3/CH_3OH/CH_3COOH/H_2O$ (25 : 15 : 4 : 2, v/v, solvent system A) [23]. The lysophospholipid was eluted from the silica with $CHCl_3/CH_3OH$ (1 : 4, v/v).

Determination of plasmalogen content of lysophosphatidylcholine. The content of 1'-alkenyl lysophosphatidylcholine in the granular lysophosphatidylcholine preparations was determined by the method of Horrocks [24] with some minor modifications.

Lysophospholipase treatment of lysophosphatidylcholine. Isolated chromaffin granular lysophosphatidylcholine was mixed with a trace of [1-^{14}C]palmitic acid and dispersed in 10 mM phosphate buffer (pH 7.0). Samples containing 200 nmol lipid phosphorus were incubated with 0.7 mg lysophospholipase II at 37°C in 0.4 ml. The lipid extract [22] was chromatographed on silica 60 HR thin-layer plates using solvent system A [23]. The extent of deacylation was calculated from the ratios of lysophosphatidylcholine phosphorus to fatty acid radioactivity assuming the same recovery for both lipids.

Incorporation of exogenous [^{14}C]lysophosphatidylcholine in intact chromaffin granules. 1-[1-^{14}C]Palmitoyl-*sn*-glycero-3-phosphocholine (9 nmol, spec. act. 55 Ci/mol) was dried and dispersed in 1.5 ml incubation buffer by sonication. To 5 ml freshly prepared granule suspension (10.7 mg protein/ml) 1 ml of this lysophosphatidylcholine dispersion was added at 4°C. After 30 min at 4°C, the granules were isolated by centrifugation (20 000 × *g* for 12 min) and the pellet was resuspended in 5 ml incubation buffer. Aliquots from the granule suspensions and the supernatant were analysed for ^{14}C radioactivity.

Lysophospholipases action on chromaffin granules. Granules were incubated at the indicated temperatures in the presence of lysophospholipase II at a concentration of 174 μg enzyme/mg granule protein. As mentioned in the text some experiments were performed with lysophospholipase I. Conditions used in each particular case are indicated in the legends of the figures. At different times aliquots (approx. 3 mg granule protein) from the incubation mixture were directly extracted according to Folch et al. [22]. When granules were pretreated with trypsin, aliquots from these incubation mixtures were extracted according to Bligh and Dyer [25]. The chloroform extracts were applied to 0.5 mm thick silica gel H plates. Development was performed as described [23] and phospholipid spots were visualized after spraying with molybdate phosphorus reagent [26]. The silica spots of lysophosphatidylcholine and sphingomyelin were scraped for phosphorus determination. Lysophosphatidylcholine hydrolysis was calculated from the change in lysophosphatidylcholine/sphingomyelin ratio.

When radioactive lysophosphatidylcholine was used the thin-layer plates were first scanned for radioactivity (Panax Ratemeter P7973) and the spots corresponding to free fatty acid, sphingomyelin and lysophosphatidylcholine were

scraped. The fatty acid spot was scraped directly in toluene scintillation fluid for analysis of ^{14}C radioactivity; the sphingomyelin spot was analysed for phosphorus and the lysophosphatidylcholine was eluted from the silica with 15 ml $CHCl_3/CH_3OH$ (1 : 4, v/v). This eluate was analysed for ^{14}C radioactivity and for phosphorus as described by Chen et al. [27] after ashing according to Ames and Dubin [28]. From the data thus obtained the following parameters were calculated: the extent of [^{14}C]lysophosphatidylcholine hydrolysis from total ^{14}C radioactivity, the extent of total lysophosphatidylcholine deacylation from the lysophosphatidylcholine/sphingomyelin ratios and the [^{14}C]lysophosphatidylcholine specific radioactivity.

Lysis determinations. The integrity of granules during incubations was followed by light scattering measurements at 540 nm [29,30] using a Varian Techtron 635 double-beam spectrophotometer equiped with thermostated cuvettes. In preliminary experiments the extent of lysis was also evaluated by determination of soluble protein released into the incubation mixture. Under the same conditions both techniques gave identical results.

Enzyme assays. Lysophospholipase activity using micellar lysophosphatidylcholine was determined as described previously [31].

Analytical methods. Protein concentrations were determined after trichloroacetic acid (10%, w/v) precipitation of the samples and solubilization of the resulting pellets in 3% (w/v) NaOH containing 2% (w/v) sodium deoxycholate [32]. Protein was then determined as described by Lowry et al. [33], with bovine serum albumin as standard.

Phosphorus present in silica scrapings was determined according to Rouser et al. [34].

Radioactivity was measured in a Packard TriCarb model 3320 liquid scintillation spectrometer using toluene containing 0.5% PPO and 0.03% POPOP. When aqueous samples had to be counted a solution of 0.7% PPO, 0.03% POPOP, and 10% naphthalene in dioxane was used.

Results

Table I gives a comparison of the phospholipid composition of bovine chromaffin granules as reported by several research groups. In general there appears to be a good agreement about the percentage of lysophosphatidylcholine, although lower levels of this component in conjunction with increased amounts of lysophosphatidylethanolamine were presented by Dreyfus et al. [37].

The phospholipid content of our preparations, as determined from the total phosphorus recovered after two-dimensional thin-layer chromatography, amounted to 324 nmol lipid phosphorus/mg protein. This is in accordance with the values reported by others [8,17,35].

Winkler and Smith [39] and Dreyfus et al. [37] have shown that the phosphatidylcholine of bovine chromaffin granules contained about 10% plasmalogen. The first authors demonstrated by gas-liquid chromatographic analysis that lysophosphatidylcholine isolated from granules consisted for about 4% of 1-1'-alkenyl-*sn*-glycero-3-phosphocholine. We have repeated these analyses by different methods to establish how much of the lysophosphatidylcholine

TABLE I

PHOSPHOLIPID COMPOSITION OF BOVINE CHROMAFFIN GRANULES

Values are expressed as percentage of total lipid phosphorus.

Phospholipid class	Percentage					
Lysophosphatidylcholine	16.8	16.8	17.4	11.8	16.7	17.4
Sphingomyelin	10.9	12.8	12.0	13.0	13.7	14.8
Phosphatidylcholine	26.0	27.5	26.3	27.0	25.4	25.3
Phosphatidylethanolamine	36.1	31.8	34.8	32.6	32.2	32.8
Phosphatidylserine	9.2	2.5	8.9	8.2	10.4	5.1
Phosphatidylinositol		8.2	1.5			2.4
Lysophosphatidylethanolamine				6.2		2.4
Phosphatidic acid	0.6	1.0		0.7	1.6	
Cardiolipin						
Data from reference	8	35	36	37	38	*

* This article. Mean values of duplicate analyses showing less than 5% variation are given.

could potentially be deacylated by lysophospholipases. Samples containing 200 nmol of isolated chromaffin granular lysophosphatidylcholine were incubated at 37°C with a large excess of lysophospholipase II (0.7 mg; spec. act. 600 nmol · min^{-1} · mg^{-1}). A plateau was reached after 94% of lysophosphatidylcholine had been hydrolysed. In a blank experiment without enzyme less than 2% were hydrolysed. 1'-Alkenyl-groups in the isolated lysophosphatidylcholine were determined by the HCl cleaving method described by Horrocks [24]. In three separate analyses 8, 9 and 12% of the total lysophosphatidylcholine were cleaved by HCl. In a control in which the HCl treatment was omitted no cleavage was found to occur. These data set the maximal amount of plasmalogen lysophosphatidylcholine derivative at 6—10%.

As can be seen in Fig. 1A, incubation of chromaffin granules at 37°C resulted in appreciable loss of membrane integrity with 30% of lysis after a 6-h period. It can also be seen that enzyme-treated granules were not more susceptible for lysis than controls. The time-course of lysophosphatidylcholine hydrolysis by added lysophospholipase (Fig. 1B) demonstrates that the percentage of lysophosphatidylcholine availability is almost identical to the value of granule lysis. This suggests that progressive granule lysis is largely responsible

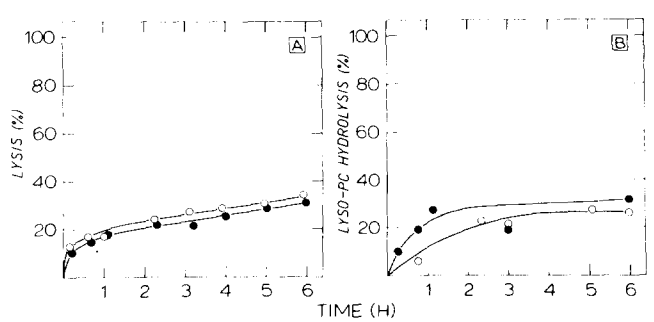

Fig. 1. Action of lysophospholipase on chromaffin granules. A suspension of intact granules (9.4 mg protein per ml) was incubated with (●———●) or without (○———○) lysophospholipase II at 37°C. A, lysis of granules; B, hydrolysis of lysophosphatidylcholine (lyso-PC).

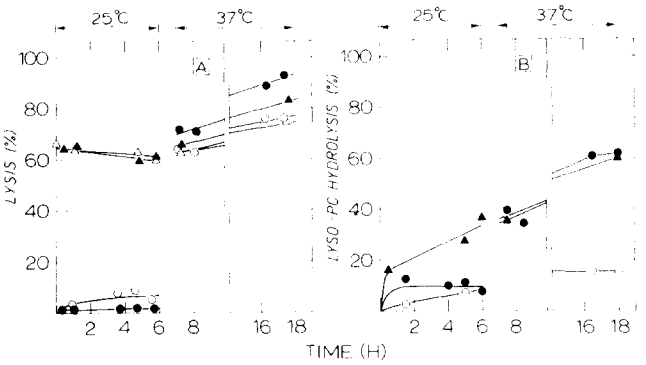

Fig. 2. Action of lysophospholipase on chromaffin granule preparations. A preparation of intact granules containing 14.1 mg protein/ml was incubated at 25°C with (●——●) or without (○——○) lysophospholipase II. Part of the granule preparation was subjected to eight cycles of freezing (—80°C) and thawing (37°C) prior to starting the incubation at 25°C in the presence (▲——▲) or absence (△——△) of lysophospholipase II. After 6 h at 25°C the incubation mixtures containing the disrupted granules (triangles) were kept in ice for 1 h. During this period the mixtures with intact granules (circles) were subjected to eight cycles of freezing and thawing. All mixtures were then further incubated at 37°C for the indicated time periods without any further additions. A, lysis of granule preparations; B, lysophosphatidylcholine (lyso-PC) deacylation in granule preparations.

for the increased lysophosphatidylcholine availability. To test this hypothesis incubations were carried out at 25°C to reduce lysis. Fig. 2A shows that 97% of granules remained intact during a 6-h treatment with lysophospholipase at 25°C. Under these conditions maximally 10—12% of lysophosphatidylcholine could be hydrolysed (Fig. 2B), suggesting that only about 10% of the acyl-lysophosphatidylcholine is present in the outer monolayer. When a preparation of disrupted granules (65% lysis, Fig. 2A) was used almost 40% of the lysophosphatidylcholine was deacylated, but a plateau in the hydrolysis process was not reached at 25°C. Ultimately, 60% of the lysophosphatidylcholine in the lysed granule preparation was deacylated upon further incubation at 37°C for an additional period of 11 h (Fig. 2B). At the end of this period lysis had increased to 83% (Fig. 2A).

In the same experiment the still largely intact granules, were subjected to eight cycles of freezing (—80°C) and thawing (+37°C) after the incubation with and without lysophospholipase for 6 h at 25°C. Determination of lysophospholipase activity with micellar lysophosphatidylcholine as substrate before and after the thermal shocks showed that this procedure had not diminished the enzymic activity. As can be seen in Fig. 2A the freezing and thawing resulted in 70% lysis for the granules incubated with lysophospholipase and this value increased to 90% during the additional incubation for 11 h at 37°C. These long incubation times did not result in much increase in the deacylation of lysophosphatidylcholine in the controls. However, a large increase in the extent of lysophosphatidylcholine hydrolysis was observed in the incubation containing lysophospholipase. Again, only 60% of lysophosphatidylcholine could be degraded, despite the fact that 80—90% of the granules had been lysed. The same value for hydrolysis was reached independently of whether disrupted or intact granules were incubated initially at 25°C.

In summary, the experiments depicted in Fig. 2 show that less than 10% lysophosphatidylcholine is available for hydrolysis in intact granules. The results with broken granules suggest that this is due to the fact that most of the lysophosphatidylcholine is localized in the inner monolayer. However, other possibilities, e.g., shielding of lysophosphatidylcholine by membrane proteins, have to be considered. In order to investigate these possibilities two types of experiments were carried out. First, the availability of a trace amount of radioactive lysophosphatidylcholine added to, and presumably incorporated into the outer monolayer of, intact granules was investigated. Secondly, the effect of pretreatment of intact granules with trypsin on the accessibility of lysophosphatidylcholine was studied.

When isolated chromaffin granules were incubated with a trace amount of 1-[1-^{14}C]palmitoyl-*sn*-glycero-3-phosphocholine 93% of the radioactivity appeared to be associated with the re-isolated granules. This amount represented 0.3% of the endogenous lysophosphatidylcholine of the granules and did not influence the extent of granule lysis. As can be seen in Fig. 3A the [^{14}C]-lysophosphatidylcholine is almost completely (91%) available for hydrolysis by bovine liver lysophospholipase II. When the specific radioactivity of the remaining lysophosphatidylcholine is plotted versus time (Fig. 3B) a rapid decrease is observed. This indicates that the radioactive tracer did not mix completely with the endogenous pool(s) of lysophosphatidylcholine and that it did not perturb the native membrane structure in the sense that the endogenous pool(s) became now accessible. It can be calculated from the data in Fig. 3 that the accessibility of the endogenous lysophosphatidylcholine in this experiment is comparable to that found in Fig. 2. Only about 10% of the endogenous lysophosphatidylcholine is available under conditions where more than 90% of the [^{14}C]lysophosphatidylcholine associated with the granules is hydrolysed. Essentially the same results were obtained when the granules, after addition of [^{14}C]lysophosphatidylcholine, were pre-incubated for 4 h at 25°C before addition of enzyme. This suggests that there is no transbilayer movement of the added [^{14}C]lysophosphatidylcholine during this time period.

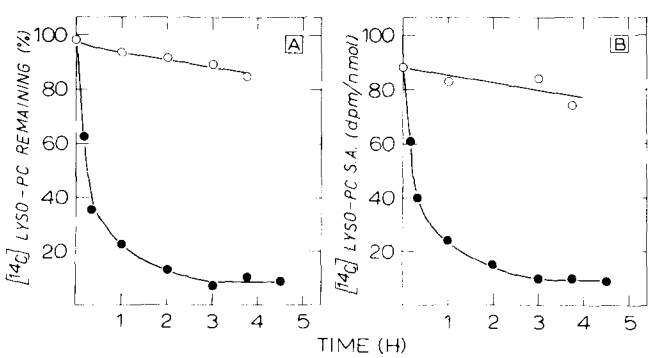

Fig. 3. Comparison of the accessibility of added and endogenous lysophosphatidylcholine in chromaffin granules. Granules were pre-equilibrated with an aqueous dispersion of 1-[1-^{14}C]palmitoyl-*sn*-glycero-3-phosphocholine. The reisolated granules were incubated with (●———●) or without (○———○) lysophospholipase II at 25°C. At the indicated times the incubation mixtures were analyzed for remaining [^{14}C]-lysophosphatidylcholine (A) and [^{14}C]lysophosphatidylcholine specific radioactivity (B).

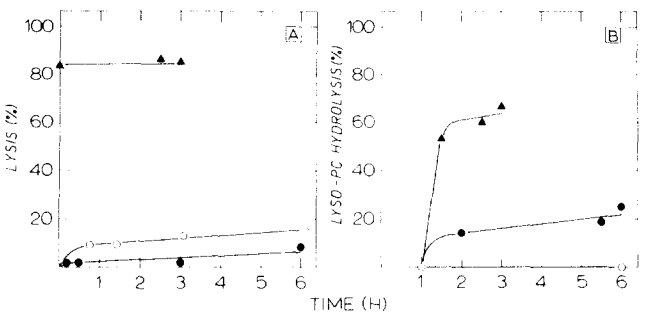

Fig. 4. Effect of trypsin-treatment of granules or ghosts on lysophosphatidylcholine accessibility. Intact granules (5.7 mg protein/ml) were incubated with (●———●) or without (○———○) trypsin (100 μg/mg granule protein) for 30 min at 25°C. The reaction was stopped by addition of trypsin-inhibitor (3 mg/mg trypsin). After further incubation for 30 min at 25°C to completely inhibit trypsin action, lysophospholipase II was added to the trypsin-treated granules. The mixtures were then incubated for the indicated time periods up to 5 h at 25°C. Essentially the same procedure was followed for granules which had been disrupted by freezing and thawing (▲———▲), with the exception that all incubations were done at 37°C and that lysophospholipase treatment was reduced to 2 h. A, lysis of granule preparations; B, lysophosphatidylcholine (lyso-PC) deacylation.

Fig. 4 shows the effect of trypsin pretreatment on the integrity of the granules and on the lysophosphatidylcholine accessibility. Although trypsin released approximately 30% of total granule protein (data not shown) this had only slight effects on lysis or lysophosphatidylcholine accessibility. This indicates that lysophosphatidylcholine protection in intact granules is not likely due to shielding by outer membrane proteins. This conclusion is fully consistent with the data from Fig. 3 which showed that lysophosphatidylcholine present in the outer monolayer is almost completely accessible to the lysophospholipase. As is apparent from Fig. 4B this granule preparation was devoid of the low lysophospholipase activity associated with the other preparations (Figs. 1B, 2B and 3). This activity probably originates from a small microsomal contamination [40].

Since the experiment in Fig. 2 had shown that lysophosphatidylcholine in disrupted granules could be hydrolysed for only 60%, the possibility that lysophosphatidylcholine present in the inner monolayer is, at least partially, shielded by proteins was also investigated by treatment of ghosts with trypsin. In agreement with the earlier experiments about 35% of the lysophosphatidylcholine remained intact when trypsin-treated ghosts were incubated at 37°C with lysophospholipase II. In this respect it is worth mentioning that the lysophospholipase activity remained constant throughout the incubation period. Thus, enzyme inactivation, due either to deficient trypsin inhibition or to granular ascorbic acid autooxidation [41,42] is not the cause of the incomplete lysophosphatidylcholine hydrolysis in ghosts.

It has previously been shown [43,44] that bovine liver lysophospholipase I (M_r 25 000) has a higher activity towards membrane-bound lysophosphatidylcholine than lysophospholipase II (M_r 60 000). Some of the experiments have been repeated with lysophospholipase I (data not shown). Despite the different properties of the lysophospholipases identical results were obtained. This provides an additional argument that the lysophosphatidylcholine accessibilities

are governed by the localization of this component in the structure of the chromaffin granule membrane rather than by the tools used.

We now consider the question why even in trypsin-treated ghosts (Fig. 4B) about 35% of the lysophosphatidylcholine is inaccessible. It should be noted that this ghost preparation still contained 18% of intact granules (Fig. 4A). This fact, together with the analysis of granular lysophosphatidylcholine (6—10% not susceptible to lysophospholipase action), sets the maximal hydrolysable pool in this preparation at about 75%. Most likely, part of the lysophosphatidylcholine, either in inner or outer monolayer, interacts with membrane constituents, e.g., integral membrane proteins, in such a way that it is protected against deacylation by lysophospholipases. This concept is corroborated by the observation that maximal hydrolysis of lysophosphatidylcholine (93% of total) was obtained after the native membrane structure had been disrupted by heating the granules for 5 min at 90°C.

Discussion

The data presented in this paper concerning the localization of lysophosphatidylcholine in bovine chromaffin granules are schematically summarized in Fig. 5. About 10% of the total lysophosphatidylcholine pool is located in the outer monolayer; it can be hydrolysed in intact granules by added lysophospholipases (Fig. 2B). Approximately 60% is located in the inner monolayer; this pool is only available in broken granules (Figs. 2B and 4B). About 6—10% of the total lysophosphatidylcholine pool cannot be localized by the methods employed because it has the plasmalogen structure, which is not susceptible to the tools used. Finally, another 20% cannot be definitively located in either outer or inner monolayer because it is not accessible even in trypsin-treated ghosts (Fig. 4B) although its acylester bond should in principle be hydrolysable by lysophospholipase. This was demonstrated for isolated granular lysophosphatidylcholine and for the lysophosphatidylcholine in heat-treated granules.

The presence of the major part of lysophosphatidylcholine in the inner monolayer is in line with the observations made by Voyta et al. [12], although

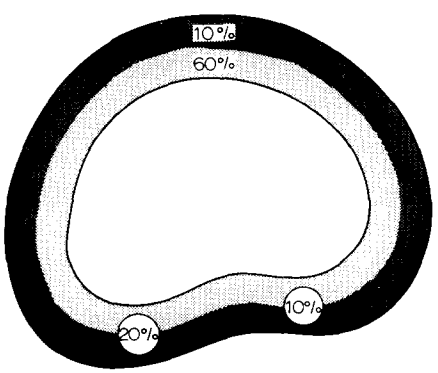

Fig. 5. Schematic representation of lysophosphatidylcholine localization in the bovine chromaffin granule membrane. Dark grey; cytoplasmic outer monolayer; light grey, inner monolayer.

our description about the localization of this membrane constituent reaches further. Voyta et al. [12] have determined how accessible the lysophosphatidylcholine was to the acyl CoA : lysophosphatidylcholine acyltransferase of rat liver microsomes. Only about 2% of acylation of lysophosphatidylcholine occurred in intact granules, but this increased markedly for broken granules. However, the highest level of acylation reported represented about one-third of the original lysophosphatidylcholine content. The quantitative difference with the accessibilities reported in this paper may be due to differences in incubation temperature, i.e., 4°C versus 25°C or 37°C used in this paper. More likely, the differences have to be explained at the level of the enzymic tools employed. Beef liver lysophopholipases I and II have been shown to attack lysophosphatidylcholine present in rat liver microsomal membranes [43,44], but also in single bilayer vesicles [13,15,43,45] and in multilayered liposomes [13,43]. The membrane-bound acyltransferase employed by Voyta et al. [12] may well be restricted in its penetration potential towards granule membranes. In this respect it is worth noting that we have recently shown that two other rat liver microsomal enzymes, i.e., phosphatidate phosphohydrolase and CTP : phosphatidate cytidylyltransferase cannot utilize phosphatidate substrate present in heat-denatured membranes [46], whereas lysophospholipases can still reach its substrate in heat-treated granules.

Some 20% of the lysophosphatidylcholine in disrupted, trypsinized granules is not accessible to lysophospholipases. In line with this observation it has been shown recently that approximately 50% of the phospholipids in chromaffin granule membranes are resistant to the action of various purified phospholipases [38]. Although the present results do not allow any conclusions concerning the nature of this protection, the increase in lysophosphatidylcholine accessibility after heat-treatment suggests that lysophosphatidylcholine-protein interactions in the native membrane are likely to be involved in the protection phenomenon. A similar effect was observed in other cases [47—49].

Exogenous 1-[1-^{14}C]palmitoyl-*sn*-glycero-3-phosphocholine equilibrated only slowly with the protected pool of lysophosphatidylcholine, in intact chromaffin granules, as revealed by lysophospholipase treatment (Fig. 3). If the protection in intact granules is caused by localization in the inner monolayer of the membrane, as we propose, it can be concluded that the transmembrane movement of lysophosphatidylcholine is slow. A slow transbilayer movement of lysophosphatidylcholine was also detected in liposomal membranes [13,14]. If the protection is due to binding by membrane proteins, a slow exchange between bound and free lysophosphatidylcholine in the membrane takes place.

In chromaffin cells lysophosphatidylcholine has been implicated in the regulation of cyclic nucleotide levels following the observations that it is able to activate guanylate cyclase [50] and inhibit adenylate cyclase [51] in adrenal medullary plasma membranes.

The possibilities for direct involvement of lysophosphatidylcholine in (physiological) membrane fusion have been recently discussed in detail [52—55]. The finding that most of the available lysophosphatidylcholine is embedded in the inner monolayer of bovine chromaffin granule membranes makes a role of this component in initiating the fusion process during exocytosis less likely. Although our in vitro experiments suggest that transbilayer movement

of lysophosphatidylcholine in intact granules is an extremely slow process, the possibility that the mechanism which triggers exocytosis induces membrane (lyso)phospholipid rearrangements, thereby facilitating the fusion process, cannot be excluded.

Acknowledgements

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