Biochimica et Biophysica Acta, 603 (1980) 336—346

BBA 79013

INTERACTIONS OF PHOSPHOLIPID VESICLES WITH RAT HEPATOCYTES IN VITRO

INFLUENCE OF VESICLE-INCORPORATED GLYCOLIPIDS

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(Received July 23rd, 1980)

Key words: Liposome; Glycolipid; Carboxyfluorescein; Phosphatidylcholine; Cerebroside effect; Lectin; (Hepatocyte)

Summary

We examined the interaction of glycolipid-containing phospholipid vesicles with rat hepatocytes in vitro. Incorporation of either *N*-lignoceroyldihydrolactocerebroside or the monosialoganglioside, G_{M1}, enhanced liposomal lipid uptake 4—5-fold as judged by the uptake of radioactive phosphatidylcholine as a vesicle marker. Cerebroside enhanced phospholipid uptake only when incorporated into dimyristoyl, but not into egg phosphatidylcholine vesicles. The lack of cerebroside effect in egg phosphatidylcholine-containing vesicles appeared to be due to a limited exposure of the carbohydrate part of the glycolipid as suggested by the reduced agglutinability of those vesicles by *Ricinus communis* agglutinin.

In contrast to the results with radioactive phosphatidylcholine, we observed only a 20% increase in vesicle-cell association as a result of glycolipid incorporation, when a trace amount of [^{14}C]cholesteryloleate served as a marker of the liposomal lipids or when using the fluorescent dye, carboxyfluorescein, as a marker of the aqueous space of the vesicles. By the same token, intracellular delivery of vesicle-contents was only slightly enhanced (approx. 10%).

The discrepancy between the association with the cells of phosphatidylcholine on the one hand and cholesteryoleate or entrapped marker on the other suggests different mechanisms of uptake for these markers. Our results are

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compatible with the notion that the main effect of incorporation of glycolipids into the vesicles is the enhancement of exchange or transfer of phospholipid molecules between vesicles and cells. Incubation of the cells with galactose or lactose, prior to addition of vesicles, suggests that this enhanced phospholipid exchange or transfer involves specific recognition of the terminal galactose residues of the glycolipid vesicles by a receptor present on the plasma membranes of hepatocytes.

Introduction

One way to achieve selective delivery of phospholipid vesicles and their contents to specified target tissues or cells is to modify properly the liposomal surface in a way which will allow specific recognition of the liposomes by the cells. Variations in parameters such as size, charge and fluidity of lipid vesicles have been shown to determine the site and mode of interaction with tissues only to a minor extent [1—4].

The demonstration of a lectin-like molecule on the plasma membrane of liver parenchymal cells which specifically recognizes terminal galactose units of desialylated glycoproteins [5,6] offered a promising opportunity for specific targetting of phospholipid vesicles to rat hepatocytes. Incorporation into the lipid bilayer of molecules carrying terminal galactose residues was likely to result in a specific interaction with their receptors. In this way the avidity of spleen and liver macrophages for intravenously injected liposomes [7—9] could hopefully be circumvented.

In two recent studies [10,11] it was shown that the presence of terminal galactose residues at the liposomal surface enhanced the association of aqueous vesicle markers with whole liver tissue in vivo. Both studies revealed an involvement of the specific galactose receptor in the uptake process. Bussian and Wriston [12] demonstrated an increased association of liposomal lipid with cultured HeLa cells incubated with glycolipid-containing phospholipid vesicles.

In this study we investigated the interaction of glycolipid-containing phospholipid vesicles with rat hepatocytes in vitro. Thus, we avoided the complication of the possible involvement of other (liver) cell types as in the in vivo studies. In addition, we investigated the nature of the interaction by discriminating between adsorption of vesicles at the cell surface and processes leading to transfer of the vesicle contents into the cell interior.

We found that incorporation of lactosylceramide or the ganglioside, G_{M1}, into phospholipid vesicles consisting of phosphatidylcholine, cholesterol and dicetyl phosphate, leads to an enhanced exchange or transfer of phosphatidylcholine molecules between vesicles and cells and to an increased binding of phospholipid vesicles to the cell surface of rat hepatocytes, but not to a concomitant increase in the transfer of vesicle-entrapped carboxyfluorescein [13,14] into the cell interior.

Materials and Methods

Materials

Egg yolk phosphatidylcholine, cholesterol and dicetyl phosphate were from

Sigma. Dimyristoyl phosphatidylcholine was purchased from Calbiochem. The monosialoganglioside, G_{M1}, was isolated from bovine brain ganglioside (Sigma) and purified by thin-layer chromatography [15]. Lactosylceramide (*N*-lignoceroyl-DL-dihydrolactocerebroside) was a product from Miles Laboratories. Egg [*Me*-^{14}C]phosphatidylcholine (49 μCi/μmol) and [*Me*-^{14}C]dimyristoyl phosphatidylcholine (45 μCi/μmol) were prepared as described by Stoffel [16]. Cholesteryl[1-^{14}C]oleate (50 μCi/μmol) was from the Radiochemical Centre, Amersham (U.K.). All lipid preparations were routinely checked for the presence of degradation products and, if necessary, repurified by thin-layer chromatography on silica gel.

Ricinus communis agglutinin (RCA-120) was bought from Miles Yeda, Ltd. Carboxyfluorescein was a product of Eastman Kodak and purified according to the method of Blumenthal et al. [17].

Methods

Small unilamellar vesicles consisting of phosphatidylcholine, cholesterol and dicetyl phosphate (and G_{M1} or lactosylceramide) at a molar ratio of 1 : 1.5 : 0.22 (: 0.3), were prepared by probe sonication as previously described [14]. When appropriate, a trace amount of [^{14}C]cholesteryloleate was included in the lipid mixture (1 mol%; see figure legends). Carboxyfluorescein-containing vesicles were prepared by the addition of an aqueous solution of 100 mM carboxyfluorescein (dissolved in aqua-bidest adjusted to pH 7.4 by addition of NaOH) to the dried lipid film and subsequent sonication. Non-entrapped and entrapped carboxyfluorescein were separated on Sephadex G-100. At the dye concentration used, the fluorescence of carboxyfluorescein is strongly quenched [13]. As has been outlined previously [14], self-quenching is relieved upon dilution. Thus, we can discriminate between, on the one hand, transfer of the aqueous vesicle contents to the cell interior resulting in vast dilution of carboxyfluorescein (and thus in fluorescent signal) and, on the other hand, binding of vesicles to the cell surface or (possibly) incorporation of vesicles by way of an endocytic mechanism, yielding fluorescent response only after addition of Triton X-100. Fluorescence measurements were carried out in a Perkin-Elmer MPF 43 fluorescence spectrophotometer with excitation and emission wavelengths of 490 and 520 nm, respectively. (Further details of the carboxyfluorescein method are described elsewhere [14,18]).

Rat liver parenchymal cells were isolated, cultured and incubated with vesicles, either in maintenance culture or in suspension culture, as previously described [19].

Lectin-induced agglutination of glycolipid-containing phospholipid vesicles was measured as described by Curatolo et al. [20], and binding of agglutinin to the lipid vesicles was determined according to the method of Surolia et al. [21]. Temperature-dependent agglutination was carried out in 1 cm light-path cuvettes fitted in a thermostatically controlled sample holder.

Protein was determined according to the method of Lowry et al. [22] and lipid phosphorus as described by Chen et al. [23]. Glycosphingolipid concentrations were assayed by using the method of Kisic and Rapport [24].

Results

Conditions for preferential lipid uptake of glycolipid liposomes

In preliminary experiments, we did not observe increased lipid uptake by hepatocytes (as judged by uptake of [^{14}C]phosphatidylcholine or [^{14}C]cholesteryloleate) from egg phosphatidylcholine vesicles as a result of lactosylceramide incorporation into the vesicles. In contrast, by substituting the monosialoganglioside G_{M1} for the lactosylceramide or dimyristoyl phosphatidylcholine for the egg phosphatidylcholine we observed a 4—5-fold increase in the uptake of [^{14}C]phosphatidylcholine. Under the same conditions, uptake of [^{14}C]cholesteryloleate was increased approx. 1.3-fold (Fig. 1).

If we assume an enhancement of lipid uptake from galactosyl-carrying liposomes to be mediated by the galactose receptor in the plasma membrane, it would be necessary for the galactosyl residues to protrude sufficiently from the liposomal bilayer to allow proper interaction with their receptors. Thus, we tentatively interpreted the lack of effect of lactosylceramide incorporation into egg phosphatidylcholine bilayers as an insufficient exposure of the lactosyl residue. The involvement of the galactose receptor in the enhanced phospholipid transfer to the cells was confirmed by the observation of a dose-dependent inhibition of phospholipid uptake by addition of galactose or

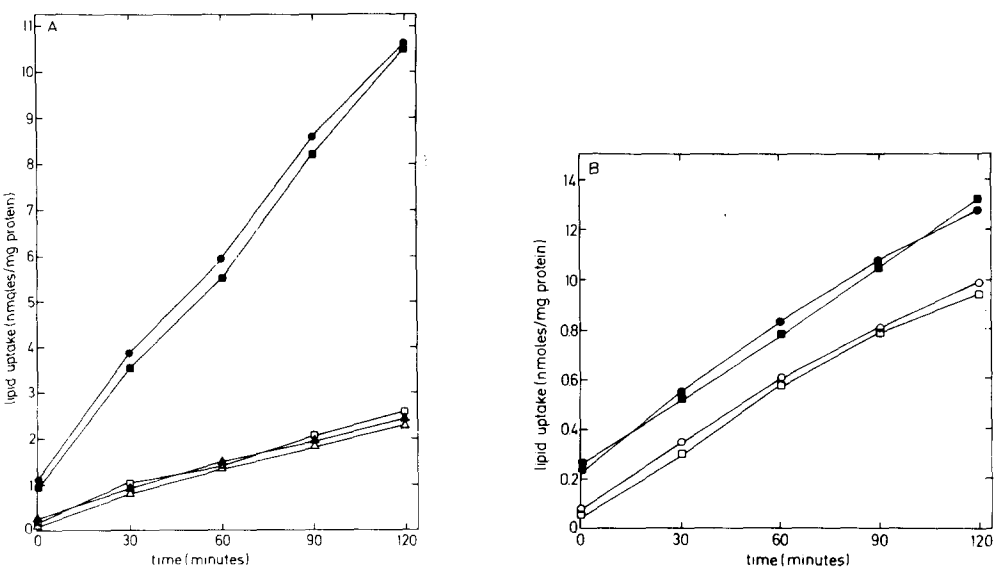

Fig. 1. Lipid uptake by hepatocytes from liposomes containing different phosphatidylcholine species with or without glycolipids. Small unilamellar vesicles (0.6 μmol lipid) were incubated with hepatocytes in maintenance culture (approx. $1.5 \cdot 10^6$ cells/dish). Lipid uptake was determined as a function of time. The markers for liposomal lipid uptake were (A) [^{14}C]phosphatidylcholine or (B) [^{14}C]cholesteryloleate. Vesicles contained: (A) egg phosphatidylcholine (△———△); egg phosphatidylcholine and lactosylceramide (▲———▲); egg phosphatidylcholine and G_{M1} (●———●); dimyristoyl phosphatidylcholine (□———□); dimyristoyl phosphatidylcholine and lactosylceramide (■———■); (B) egg phosphatidylcholine (○———○); egg phosphatidylcholine and G_{M1} (●———●); dimyristoyl phosphatidylcholine (□———□); dimyristoyl phosphatidylcholine and lactosylceramide (■———■). In addition, all vesicle preparations contained cholesterol and dicetyl phosphate. The molar ratio of phosphatidylcholine : cholesterol : dicetyl phosphate (: glycolipid) was 2 : 1.5 : 0.22 (: 0.3).

lactose to the incubation mixture (not shown).

The extent of protrusion of the galactosyl residues out of the liposomal membrane can be assessed by measuring the agglutination of the liposomes by appropriate lectins [21,25]. Conditions which will fulfil the prerequisite of sufficient carbohydrate extension can then easily be tested with this model system. By entrapment of the fluorescent dye carboxyfluorescein, we would also be able to assess any possible disruption of the bilayer structure by the lectins as a result of glycolipid rearrangements. In studies of liposome-cell interactions such as these, it is of crucial importance that liposome integrity be maintained in order to attain efficient transfer of entrapped aqueous markers into the cell interior.

We incorporated lactosylceramide into liposomes containing either egg phosphatidylcholine or dimyristoyl phosphatidylcholine and examined the availability of the terminal galactose residues by means of their binding capacity to a specific galactose-binding protein from *R. communis*.

Lectin-induced agglutination of glycolipid vesicles

As a measure of carbohydrate exposure, we determined initial agglutination rates by monitoring the absorbance at 450 nm as a function of time. The initial velocity of agglutination of dimyristoyl phosphatidylcholine/lactosylceramide vesicles increases with increasing lectin concentration (Fig. 2A) and, under the experimental conditions, maximal agglutination is attained at lectin concentrations of approx. 60 μg/ml. The dependence of initial agglutination rates on vesicle concentration is shown in Fig. 2B.

Under conditions (37°C) producing extensive agglutination of dimyristoyl phosphatidylcholine vesicles containing lactosylceramide, the rate of agglutination of glycolipid vesicles, containing egg phosphatidylcholine instead of the dimyristoyl analogue, was 5-times lower. Quantitative determination of the amounts of lectin bound by the vesicles revealed that dimyristoyl phosphatidylcholine/lactosylceramide vesicles bound 4—5-times as much as egg phosphatidylcholine/lactosylceramide vesicles (229 and 48 μg, respectively; 0.9 μmol of vesicle lipid was incubated with 0.6 mg lectin). Agglutination was

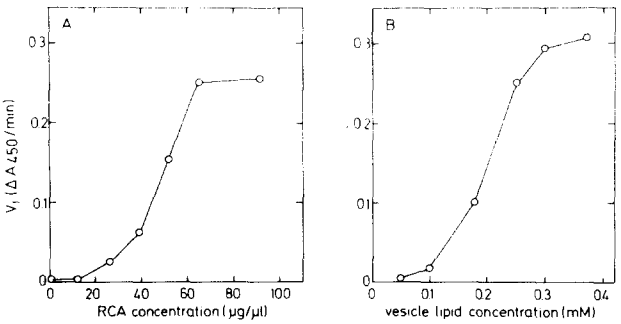

Fig. 2. Initial velocity of agglutination of glycolipid-containing vesicles as a function of (A) lectin concentration and (B) vesicle lipid concentration. Vesicles consisted of dimyristoyl phosphatidylcholine, cholesterol, dicetyl phosphate and lactosylceramide (molar ratio 2 : 1.5 : 0.22 : 0.3). The incubations were performed at 37°C in 5 mM Tris/0.135 M NaCl, pH 7.4 (final volume 2.0 ml), containing (A) 0.25 μmol/ml of lipid or (B) 65 μg/ml *Ricinus communis* agglutinin (RCA).

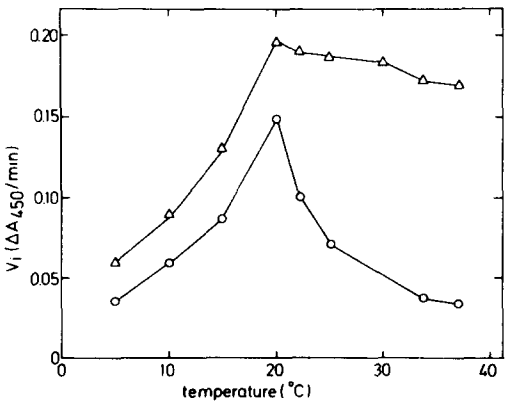

Fig. 3. Initial agglutination rates of glycolipid vesicles as a function of temperature. Vesicles consisted of dimyristoyl phosphatidylcholine (△———△) or egg phosphatidylcholine (○———○) and cholesterol, dicetyl phosphate and lactosylceramide (molar ratio 2 : 1.5 : 0.22 : 0.3). Incubation conditions were as described in the legend of Fig. 2.

strongly dependent on temperature (Fig. 3). Dimyristoyl as well as egg phosphatidylcholine vesicles (both containing lactosylceramide) showed increasing agglutination rates with increasing temperature over the range 4—20°C. At approx. 20°C, maximal agglutination rates were attained. Upon increasing the temperature above 20°C, the agglutinability of the egg phosphatidylcholine/lactosylceramide vesicles steeply decreased, whereas the rate of agglutination of the dimyristoyl phosphatidylcholine/lactosylceramide vesicles decreased only very slowly.

Over the temperature range applied no increase in turbidity was observed during incubation of glycolipid vesicles in the absence of the agglutinin or of vesicles containing no glycolipid, either in the presence or absence of lectin. The limited accessibility, at 37°C, of the galactosyl residues in the egg phosphatidylcholine/lactosylceramide vesicles towards the *R. communis* agglutinin is compatible with the lack of increase in lipid uptake by hepatocytes of such vesicles as compared to vesicles without the lactosylceramide (cf. Fig. 1). This lack of stimulation might, indeed, be explained by this limited accessibility.

In order to determine vesicle integrity during glycolipid-lectin interaction, we entrapped carboxyfluorescein in dimyristoyl phosphatidylcholine/lactosylceramide vesicles. The experiments showed that during lectin-induced agglutination the integrity of the vesicles was well preserved. Irrespective of the presence of glycolipids in the vesicles and of the lectin in the medium, the rate of leakage of carboxyfluorescein from the vesicles remained at the control level of approx. 5% per h (results not shown).

Uptake of carboxyfluorescein-containing glycolipid vesicles

In order to establish whether transfer of liposomal lipid markers to the cells (cf. Fig. 1) is accompanied by transfer of the aqueous contents of the vesicles, we trapped carboxyfluorescein in phospholipid vesicles. This method (see Ref. 14 and Materials and Methods) only allows studies of initial kinetics of transfer

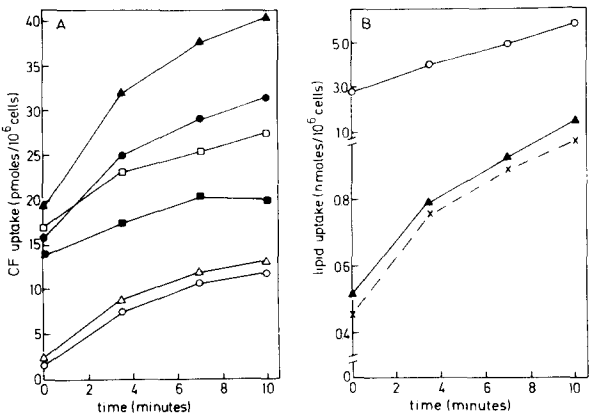

Fig. 4. (A) Uptake by rat hepatocytes of the aqueous-space marker carboxyfluorescein entrapped in vesicles with or without glycolipid. Uptake of carboxyfluorescein (CF), entrapped in vesicles consisting of egg phosphatidylcholine, cholesterol, dicetyl phosphate with or without G_{M1} (ratio as in Fig. 1) was determined as a function of time. Incubations were performed in suspension cultures containing $2 \cdot 10^6$ cells per ml (total volume 10 ml). Vesicle lipid concentration was 0.125 μmol/ml. Molar ratio of carboxyfluorescein/lipid in G_{M1} vesicles was 0.042, in vesicles without G_{M1} 0.044. At the times indicated, samples of 1 ml were taken and the fluorescence was determined before and after addition of detergent (see Materials and Methods). Uptake of carboxyfluorescein in G_{M1}-containing vesicles: △———△, calculated from fluorescence prior to addition of Triton X-100 (a); ▲———▲, calculated from fluorescence after addition of Triton X-100 (b); □———□, fraction of carboxyfluorescein, of which the fluorescence is relieved due to addition of detergent (b − a). Uptake of carboxyfluorescein in vesicles without G_{M1}: ○———○, prior to addition of detergent (p); ●———●, after addition of detergent (q); ■———■, fraction calculated from fluorescence increment after addition of detergent (q − p). (B) Discrepancy between uptake by hepatocytes of liposomal lipid markers and entrapped carboxyfluorescein from G_{M1}-containing vesicles. Vesicles (see A) contained [^{14}C]phosphatidylcholine (○———○) or 1% [^{14}C]cholesteryl oleate (▲———▲) as lipid markers. Samples were taken for radioactivity measurements and fluorescence readings and from either result lipid uptake was determined . The molar ratio of carboxyfluorescein : lipid was used to calculate lipid uptake (X———X) from the fluorescence readings. Incubation conditions were as described in A. Note the difference in scales between the upper and lower parts of the figure.

and binding for reasons indicated elsewhere [14,18]. We therefore limited our incubations to periods of 10 min. The experiments presented here were performed with egg phosphatidylcholine/G_{M1} vesicles. Dimyristoyl phosphatidylcholine/lactosylceramide vesicles gave similar results. The results (Fig. 4A) demonstrate that the amount of dye internalized by the cells is only slightly enhanced when G_{M1} is incorporated into the vesicles. Total cell-associated carboxyfluorescein is slightly more increased as a result of G_{M1} incorporation. The difference between total cell-associated dye and transferred dye represents a fraction of vesicles in which carboxyfluorescein is still present at a high concentration and of which the self-quenching is only relieved upon addition of detergent.

This fraction could have a dual origin: vesicles attached to the cell surface or internalized vesicles in which the dye is still present at a high concentration, i.e., endocytic vacuoles and/or lysosomes. The presence of metabolic inhibitors (50 mM sodium deoxyglucose and 5 mM NaN_3) during vesicle-cell interaction had no effect on the magnitude of this quenched fraction (cf. Ref. 14). This would argue against the involvement of energy-dependent endocytosis in the mechanism of uptake. Addition of 100 mM galactose to the cells prior to addition of vesicles reduced both binding (approx. 15%) and transfer (5—19%)

of carboxyfluorescein entrapped in G_{M1} vesicles, whereas control vesicles were unaffected.

It should be noted that the limited effect of the galactose on binding is in agreement with the rather small increase in binding that we find as a result of G_{M1} incorporation (compare open and filled squares in Fig. 4A).

We conclude that, although incorporation of glycolipids in appropriate phospholipid vesicles does result in enhanced association of a marker of the aqueous space with hepatocytes, this enhancement is primarily due to an increased attachment of vesicles to the cell surface.

In Fig. 4B, a comparison is made between values for total vesicle lipid uptake calculated either from the carboxyfluorescein measurements or from the uptake of the lipid markers, phosphatidylcholine and cholesteryloleate. Uptake based on cholesteryloleate closely resembled the uptake based on carboxyfluorescein, whereas the uptake based upon [^{14}C]phosphatidylcholine is 5—6-fold higher. Qualitatively, these results are compatible with those observed in a previous study [14]. In that study we reported on the interaction of hepatocytes with vesicles not containing glycolipids and presented evidence of the occurrence of extensive exchange of phosphatidylcholine between vesicles and hepatocytes. Presumably, the fate of the phosphatidylcholine in the present study is similar, i.e., the incorporation of glycolipid enhances the exchange of the phospholipid between vesicles and cells, without leading to an increased transfer of entrapped solute. The high zero-time value of phosphatidylcholine uptake, which is also observed for the G_{M1}-containing vesicles in Fig. 1, emphasizes that we are dealing with a rapid process: within the time required to achieve complete separation of cells and vesicles a very considerable amount of phospholipid has become cell associated.

Discussion

In previous studies [14,26] in which we used vesicles without glycolipids, binding of vesicles and exchange of phosphatidylcholine were found to contribute to a considerable extent to the processes by which liposomal lipid becomes associated with isolated hepatocytes. The present study indicates that also the liposomal constituents, which become additionally hepatocyte associated as a result of the incorporation in the vesicle bilayer of ligands for the plasma membrane galactose receptor [27], represents mainly transfer of individual phosphatidylcholine molecules and, to a limited extent, association of whole vesicles.

The stimulatory effects of glycolipid incorporation which we observe may be an underestimation of the actual capacity of the hepatocytes to interact with such liposomes because proteolytic enzymes present in the collagenase used for the isolation of the cells might have digested receptor sites [27].

Two recent studies focused on the use of glycolipid to enhance liposomal uptake [11,12]. Surolia and Bacchawat [11] observed an enhanced association of liposome-entrapped invertase with liver tissue when G_{M1}-containing vesicles were injected intravenously into rats. Although the authors did not ascertain the intrahepatic cellular localization of the enzyme, they suggested that the uptake was mediated by the receptor present on the plasma membrane of

hepatocytes. However, their results do not exclude enhanced binding of vesicles as an alternative for internalization of liposomal contents. The decrease in liver-associated invertase starting about 30 min after injection of invertase-containing G_{M1} liposomes (cf. Fig. 1 in Ref. 11) may partly be explained by a release of enzyme from cell surface-adsorbed liposomes. We observed similar phenomena with isolated hepatocytes during prolonged incubation with carboxyfluorescein-containing G_{M1} vesicles. In a separate study we found that the loss of fluorophore results in part from leakage of the dye from vesicles which are bound to the cell surface [14].

Bussian and Wriston [12] showed an increase in the association of liposomal lipid with HeLa cells as a result of incorporation of lactosylceramide into the vesicles and ascribed this to enhanced fusion of the vesicles with the cells. Exchange or transfer of individual lipid molecules as well as mere adsorption of vesicles, however, cannot be excluded as alternative interpretations of their results as identical behavior of phospholipid and cholesterol label are no proof of fusion. In addition, the incorporation of lactosylcerebroside in their experiments may have led to quantitatively different influences on phosphatidylcholine and cholesterol exchange.

The results presented in Fig. 1 show that enhanced lipid uptake from glycolipid-containing vesicles by the cells depends on the type of vesicle used. These differences are likely to be related to variations in the extent of carbohydrate exposure. The exposure of ligand residues at the liposomal surface is known to be influenced by several variables: carbohydrate chain length [29] and mobility of the glycolipid in the plane of the bilayer [30,31], fatty acid chain length of the phospholipid [24] and the presence of cholesterol in the liposomal bilayer [29,31].

The carbohydrate moiety of a glycolipid containing several sugar residues (G_{M1} as compared to lactosylceramide) will presumably protrude sufficiently far from the hydrocarbon region to keep it from being embedded in the polar region of the (egg)phosphatidylcholine bilayer (Fig. 1 and Ref. 29). In addition, the sialic acid residue in G_{M1} may contribute to an enhanced accessibility of the terminal galactose residues, although removal of the sialic acid was claimed not to affect hepatic uptake of G_{M1}-containing liposomes [11].

The accessibility of lactosylceramide galactose residues to the *Ricinus* agglutinin was shown to depend on the phospholipid species in the bilayer and on temperature, as illustrated in Fig. 3. Our finding that agglutination at 37°C of egg phosphatidylcholine/lactosylceramide vesicles is markedly reduced as compared to dimyristoyl phosphatidylcholine/lactosylceramide vesicles provides an explanation for our failure to demonstrate an increase in cellular uptake of egg phosphatidylcholine as a result of lactosylceramide incorporation into such vesicles. The temperature-dependent agglutination profile for the egg phosphatidylcholine/lactosylceramide system appeared to be very similar to that observed by Curatolo et al. [20]. These authors suggested conformational changes of the lectin to be the main cause of a reduced agglutinability at temperatures exceeding 25°C. Our results suggest this to be unlikely, since dimyristoyl phosphatidylcholine/lactosylceramide vesicles still show extensive agglutination above this temperature. We believe, therefore, that variable exposure of galactose residues causes the temperature

dependence of vesicle agglutinability rather than conformational changes of the lectin. The relatively short acyl chain in dimyristoyl phosphatidylcholine vesicles probably allows the galactose residue to remain sufficiently exposed, even at higher temperatures, to show agglutinability (Fig. 3) and interaction with the plasma membrane receptor (Fig. 1). Galactose exposure in glycolipid vesicles is enhanced by incorporation of cholesterol [33] and, according to our experiments, it decreases when the temperature increases above 20°C with the liposome composition we used. Possibly, in this temperature range, the glycolipid preferentially diffuses into regions enriched in phospholipid as a result of lateral phase separations in the bilayer [34]. This would decrease the extent of galactose exposure in egg phosphatidylcholine vesicles, while in the shorter-chain dimyristoyl phosphatidylcholine vesicles galactose exposure and thus agglutinability might still occur. It is also conceivable that other temperature-induced structural changes at the glycolipid/water interface cause variations in polar headgroup conformation. Tilt changes in the hydrocarbon chains of the glycolipid have been reported by Dahlen and Pascher [35]. A recent report by Bunow and Bunow [37] on the phase behavior of ganglioside/phosphatidylcholine mixtures possibly argues in favor of a phase transition in the G_{M1} causing the abrupt changes in agglutinability at 20°C, as shown in Fig. 3. The immiscibility of the ganglioside with dimyristoyl phosphatidylcholine as reported by the same authors [37] would then explain the difference in slope of the rates of agglutinability above 20°C. It is obvious that with the scanty knowledge that exists of the physico-chemical properties of glycolipids and their mixtures with other membrane lipids (cf. Refs. 36 and 37), the interpretations of our observations are, by necessity, speculative. Nonetheless, the correspondence we found between lectin agglutinability of glycolipid vesicles and their behavior towards cells shows that this approach may provide a useful contribution to the study of the interaction of vesicles with receptors on cell membranes.

Acknowledgements

We thank Mrs. Karin van Wijk and Mrs. Rinske Kuperus for competent secretarial work. These investigations were carried out under the auspices of the Netherlands Foundation for Medical Research (FUNGO) with financial support from the Netherlands Organization for the Advancement of Pure Research (ZWO).

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