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Modulation of red blood cell sugar transport by lyso-lipid

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The in vitro presentation to red blood cells of specific lysolipids in amounts comparable to lysolipid levels in serum is shown to markedly influence protein-mediated glucose transport. Lysolipids were introduced exogenously into cell membranes by incubating erythrocytes in buffer containing varying concentrations of lysolipid (under 3.2 μM). The transport-modulating potency of the lysolipids was found to be dependent both on headgroup and hydrocarbon chain. MPL (monopalmitoyl lecithin, L-α-lysopalmitoylphosphatidylcholine) had the greatest influence on sugar transport. 15 min incubation of red cells in MPL suspensions sufficed for 99% association of the lysolipid with the cell membranes. This association correlated with altered red-cell sugar transport. At MPL/bilayer lipid molar ratios as low as 0.03%, MPL was found to act as a reversible, hyperbolic, mixed-type inhibitor of exchange D-glucose exit (both $K_{m(app)}$ and V_{max} for transport are reduced). Dissociation of MPL from the membrane results in the recovery of original transport activity. MPL at $1.5 \cdot 10^{-17}$ mol MPL/red cell was found to reduce $K_{i(app)}$ for D-glucose inhibition of cytochalasin B binding to the glucose carrier protein in red cell ghost membranes. Our findings demonstrate that red-cell membrane-exogenous lysolipid associations can significantly modify protein mediated sugar transport. The simplest explanation of our findings is a direct interaction of lysolipid with the transport protein.

Introduction

Many membrane processes are sensitive to the presence of small amounts of specific bilayer lipids. One class of such active lipids is the lysolipids. Lysolipids are soluble in lipid bilayers [1] and most likely exert their influence on membrane enzymes through the bilayer phase. The effects of lysolipids on membrane activities are diverse with respect to the membrane activity effected and specific with regard to the active lysolipid species.

Lysolipids capable of altering particular membrane functions are present in vivo both in normal and pathological situations. Microsomal glucose-6-phosphatase from rat liver is specifically and reversibly inhibited by lysophosphatidylcholine in intact bilayers [2], while rat liver phenylalanine hydroxylase is activated by lysophosphatidylcholine [3]. The Na^+/K^+-ATPase activities of canine kidney cortex and of the supraorbital salt glands of ducks are found to be inhibited by lysolipids at concentrations found in normal human plasma [4]. These latter inhibitions are similar to those found in such human diseases as essential hypertension and chronic renal insufficiency [4]. Similarly, the Na^+/K^+-ATPase activity and ouabain-binding capacity of the hog erythrocyte are strongly inhibited by the low amounts of γ-acyllysophospholipids found in the plasma of volume-expanded animals [5].

Exposure of isolated canine Purkinje fibers to lysolipid levels present in the effluents of anoxic isolated rabbit hearts or accumulated in perfused heart and myocardium rendered ischemic in situ results in altered action potentials resembling those seen in ischemic myocardium in vivo. It appears that accumulation of lysolipids induced by ischemia may contribute to the genesis of malignant dysrhythmia early after its onset [6]. Similarly, lysolipids introduced at concentrations equivalent to those found in ischemic feline myocardium induced electrophysiological dearrangement in vitro resembling those typical of ischemic tissue in vivo [7].

Low amounts of lysolipids are found in most biological membranes. In part, this no doubt reflects their role as intermediates in metabolism. Various membrane-

Abbreviations: BSA, bovine serum albumin; CMC, critical micellar concentration; LPE, lysophosphatidylethanolamine; LPS, lysophosphatidylserine; MML, monomyristoyl lecithin; MOL, monooleoyl lecithin; MPL, monopalmitoyl lecithin; MSL, monostearoyl lecithin; MMPE monomyristoylphosphatidylethanolamine; PC, phosphatidylcholine; PLA_2, phospholipase A_2.

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bound phospholipases have been identified in biomembranes [1]. Phospholipase A_2 activity appears to play a role in receptor activation in cellular signalling [8]. Lysolipid formation in arachidonate metabolism is known to play a role in cellular signalling [9].

In the human red blood cell, lysolipids normally comprise several percent total membrane phospholipids [10]. Since serum lysolipid is readily exchangeable with the red blood cell membrane [6,11], the demonstration that small increases in membrane lysolipid content from exogenous sources can inhibit sugar transport would suggest that serum lysolipids may be a factor in modulating membrane processes in vivo. In Type II hyperlipoproteinemia, for example, the lysolipid carried by α-lipoproteins can be double that of normal individuals [12].

In previous investigations, we characterized in detail how different lipid types influence the activity of the reconstituted human erythrocyte sugar transporter [13–17]. It was demonstrated that sugar transporter activity was strongly dependent on the bilayer's overall lipid composition and physical state (for reviews, see Refs. 18,19). As an extension of these studies, we extended our work to determine how various exogenously introduced lysolipids affected protein-mediated sugar transport in the human erythrocyte. We find that very low levels of lysolipids, in particular monopalmitoyl lecithin (MPL, 1-palmitoyl-*sn*-glycero-3-phosphatidylcholine) – the most common lysolipid found in the red blood cell membrane, significantly modulates sugar transport in the native-red-cell membrane.

Methods

Solutions. The following solutions were used: lysis solution containing 10 mM Tris-HCl and 4 mM EDTA (pH 7.2); Tris medium containing 50 mM Tris-HCl and 0.2 mM EDTA (pH 7.4); saline consisting of 150 mM NaCl, 2 mM EDTA and 5 mM Tris-HCl (pH 7.4). The pH of all solutions was adjusted using 1 mM Tris base.

Cells. Erythrocytes were collected from freshly outdated blood by centrifugation and then washed three times in saline [20]. Unsealed ghosts were formed from washed cells as described in Carruthers and Melchior [20] and resuspended in Tris medium at a concentration of 2 mg membrane protein per ml.

Equilibrium exchange efflux studies. Equilibrium exchange efflux measurements were made with intact red cells. Washed cells were loaded with sugar by suspending red cell pellets in saline containing various concentrations (5–400 mM) of unlabelled D-glucose and incubating for 1 h at 37°C. The loaded cells were collected by centrifugation. The pellet (2 ml) was brought up to 4 ml total volume with saline containing D-glucose (extracellular [D-glucose] = intracellular [D-glucose]) and ^{14}C-labelled D-glucose (5 μCi at 1.88 mCi/mmol, Pathfinder Laboratories, St. Louis, MO) was added. This mixture was allowed to incubate for 20 min at 37°C. The mixture was divided into various aliquots. For studies with lysolipids, buffer containing a concentrated suspension (carrier-free) of the appropriate lysolipid (Avanti Polar Lipids, Birmingham, AL) was added to the aliquots to yield final concentrations of $(0–3.2)\cdot10^{-6}$ M, $((0–3)\cdot10^{-17}$ mol MPL per red cell) (CMC of MPL = $2\cdot10^{-6}$ M [1]). Treatment of cells with lysolipids for transport studies was carried out for 15 min at 37°C to obtain maximum association of lysolipids with the membrane. For measurements of bilayer leakage, an appropriate amount of saline containing 100 mM $HgCl_2$ (a protein transport inhibitor) was added to give a final $HgCl_2$ concentration of 0.5 mM.

Efflux experiments were carried out on ice. These were initiated by rapidly adding 1.5 ml of ice-cold saline containing appropriate concentrations of unlabelled glucose, lysolipid and $HgCl_2$ to 25 μl of packed D-[^{14}C]glucose-loaded cells. The concentrations of D-glucose, lysolipid and $HgCl_2$ in the saline medium were identical to that contained in the aliquots of loaded cells. After the required efflux interval, fluxes were arrested by the rapid addition of 75 μl ice-cold 100 mM $HgCl_2$ followed by centrifugation (30 s). Control experiments demonstrate that sugar exit is arrested by addition of $HgCl_2$ (Fig. 1). The supernatant was aspirated, and the pellet washed in 1.5 ml stopping buffer (0.5 mM $HgCl_2$ in saline plus sucrose at a

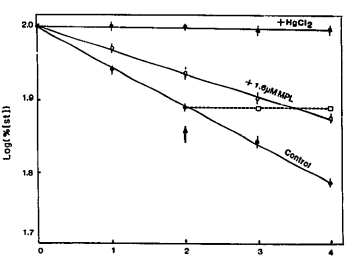

Fig. 1. The effect of monopalmitoyl lecithin (1.6 μM; $1.5\cdot10^{-17}$ mol MPL/red cell) on equilibrium exchange glucose efflux (100 mM D-glucose) from human erythrocytes. Ordinate: log of the percent of radiolabelled D-glucose remaining in the cells. Abcissa: time in minutes. The curves drawn through the points were calculated by method of least squares. Control cells (●); cells treated with monopalmitoyl lecithin (○); cells treated with 0.5 mM $HgCl_2$ (★). For one series of experiments, after 2 min of efflux, control cells were treated with 0.5 mM $HgCl_2$ (arrow) with the results shown by the dashed curve (------). Each point is the average of five separate experiments.

concentration identical to the glucose content of the cells) centrifuged and the cell pellet was disrupted with 1 ml 3% perchloric acid. The extract was centrifuged and the activity in the clear supernatant counted by liquid scintillation spectrophotometry. The rate of exchange exit (V, mmol/l per min) is obtained as the product slope 2.3 [S] from a plot of log [St] versus time where [S] = intracellular and extracellular glucose concentration and [St] is the fraction of radiolabel remaining in the cells at time t. Slopes were calculated by linear regression and rates of sugar leakage (measured in the presence of $HgCl_2$) were subtracted from control and lysolipid exit data.

Reversibility of inhibition studies. Washed cells (2 ml) were loaded with 100 mM D-glucose for 60 min at 37°C and then collected by centrifugation at 25 000 × g for 20 min. The preparations were incubated in 4 ml Tris buffer containing 100 mM D-glucose and MPL ($1.6 \cdot 10^{-6}$ M) for 15 min at 37°C. The cells were collected by centrifugation. As a control, half of the cell pellet was exposed to ^{14}C-labelled D-glucose and MPL ($1.6 \cdot 10^{-6}$ M) for 30 min. The remaining cells were diluted up to 50-fold with buffer containing 100 mM D-glucose and no MPL and were incubated for 1 h at 37°C. These cells were then collected by centrifugation and exposed to ^{14}C-labelled D-glucose for 30 min. Efflux experiments on both this and the control fraction were performed as described previously.

Cytochalasin B binding studies. D-Glucose-sensitive, cytochalasin-D- (10 μM) insensitive cytochalasin B binding was measured by incubating control and lysolipid treated cells or ghosts for 10 min at 20°C with 50 nM [^{3}H]cytochalasin B (18.5 Ci/mmol, New England Nuclear, Boston, MA), various concentrations of unlabelled cytochalasin B ±1.6 μM MPL and varying concentrations of D-glucose. Equilibrium binding is achieved in this time [22]. Samples of the suspension (20 μl) were counted as totals, then, following centrifugation 10 μl samples of the clear supernatants were counted. The difference between totals and supernatants is the specific binding of [^{3}H]cytochalasin B to the sugar transport molecules [13,21].

Association of MPL with cells and membranes. To determine the amount of MPL associated with red cells and membranes, experiments were performed with ^{14}C-labelled MPL (Amersham Corp., Arlington Heights, IL). Washed, MPL-loaded cells or ghosts were exposed to labeled MPL ($1.5 \cdot 10^{-17}$ mol MPL/cell) – for different time intervals and centrifuged for 60 s in a microfuge, and the supernatant was aspirated. Aliquots of suspension and supernatant were collected and counted prior to and following centrifugation. The pellets were resuspended and aliquots of the suspension were counted. For measurements of the dissociation of MPL from cells and ghosts, the remaining cells or ghosts (incubated with MPL for 60 min) were centrifuged, the supernatant was aspirated and then the pellets were diluted by up to 50-fold with buffer containing 100 mM D-glucose. 4-ml aliquots of this suspension were removed at different time intervals and centrifuged, and the activity was measured as described previously. Uptake of MPL by MPL-loaded cells during sugar efflux experiments at 4°C was determined by incubating 15 μl packed cells with 1 ml efflux medium containing the appropriate MPL concentration plus [^{14}C]MPL. The tubes were centrifuged for 30 s at time intervals (0–4 min) and the difference between suspension and supernatant counts calculated as bound activity. Fig. 3 shows the time-course of lysolipid association with the membrane.

Conversion values. 1 μl of packed cells is equivalent to 6 μg membrane protein. For calculations purposes, a single human red blood cell membrane is considered to contain $7.2 \cdot 10^8$ lipid molecules, 0.6 pg membrane protein [14,22] and $1.5 \cdot 10^5$ sugar transport molecules [21].

Results

Red-cell exchange D-glucose transport was significantly modified by several of the lysolipids investigated

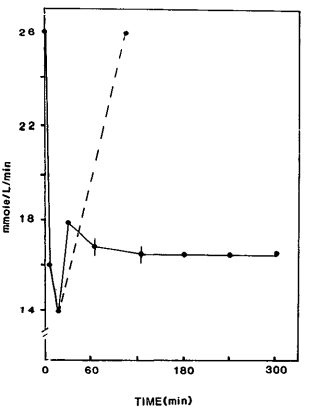

Fig. 2. The time-course of inhibition of D-glucose efflux from human erythrocytes by monopalmitoyl lecithin ($1.5 \cdot 10^{-17}$ mol MPL/red cell). Ordinate: rate of D-glucose exit at 100 mM D-glucose in mmol/l cell water per min. Ordinate: time in minutes. In one series of experiments after 15 min exposure of cells to MPL, cells were diluted 50-fold in MPL-free glucose medium and transport measured after 90 min (dashed line). Each point is the average of three separate experiments.

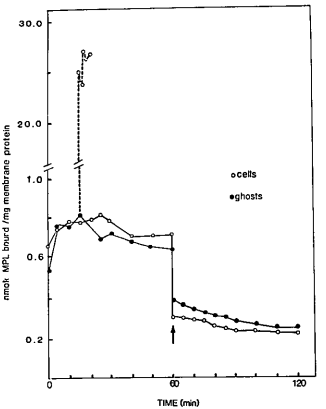

Fig. 3. The time-course of binding of monopalmitoyl lecithin to human erythrocytes (○) and cell ghosts (●). Ordinate: nmol of MPL bound per mg membrane protein. Abscissa: time, minutes. The MPL : red blood cell ratio is $4.2 \cdot 10^{-19}$ mol MPL/red blood cell. At 60 min, a portion of the cells and membranes were diluted 50-fold into MPL-free glucose medium. The dashed line represents the additional binding of MPL to cells occurring during an efflux experiment where the MPL : red blood cell ratio is $1.5 \cdot 10^{-17}$. Each point is the average of two separate experiments.

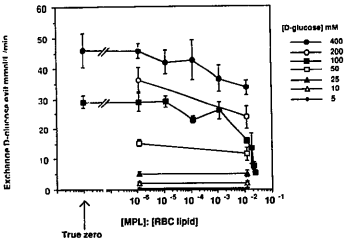

Fig. 4. The effect of different concentrations of monopalmitoyl lecithin (MPL) on glucose efflux from human erythrocytes at different concentrations of glucose; ○, 400 mM; ●, 200 mM; ■, 100 mM; □, 50 mM; ▲, 25 mM; △, 10 mM; ◆, 5 mM. Ordinate: rate of glucose exchange exit in mmol/l cell water per min. Abscissa: log molar concentration of MPL relative to red blood cell lipid. Dose-response measurements of various concentrations of MPL were made at 400 (○) and 100 mM (■) glucose. The points for 400 and 100 mM D-glucose are the averages of three experiments, the other points are the averages of two experiments.

in this study (Figs. 7, 8). The most notable of these was MPL. The association of this lipid with the red cell membrane and its effects on sugar transport were investigated in some detail. MPL at levels as low as $1.5 \cdot 10^{-17}$ mol per red cell inhibits protein-mediated D-glucose exchange exit in the human red blood cell (Fig. 1). The time-course of inhibition of D-glucose (100 mM) exist by MPL at this lysolipid : red-cell ratio is shown in Fig. 2 (solid line). Maximum inhibition is reached at 15 min exposure of cells to MPL. This time-course of inhibition of transport correlates well with the time-course of [^{14}C]MPL binding to red-cell ghosts and intact cells (Fig. 3). This inhibition is reversible. Inhibition of transport by MPL is reversed by 90 min incubation of MPL-treated cells in MPL-free medium (Fig. 2, dashed line). At this time, 50% of the MPL associated with the membrane is lost (Fig. 3). During the course of glucose exit experiments, MPL is also taken up by the cells. For 15 μl of packed, MPL-pretreated cells suspended in 1 ml of 1.6 μM MPL-saline at 4°C, as much as 87% of total MPL is taken up within 1 min.

Inhibition of equilibrium exchange D-glucose efflux by MPL displays a strong dependence upon D-glucose concentration (Fig. 4). At an MPL : red blood cell ratio of $1.5 \cdot 10^{-17}$ mol/red cell, inhibition of transport is

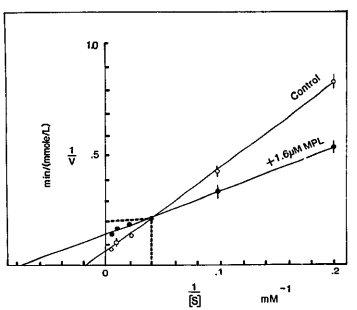

Fig. 5. Lineweaver-Burk plot of the concentration dependence of glucose efflux from human erythrocytes in the presence (●) and absence of (○) monopalmitoyl lecithin (1.6 μM MPL; $1.5 \cdot 10^{-17}$ mol MPL/red blood cell). Ordinate: reciprocal of glucose exit rate in mmol/l per min. Abscissa: reciprocal of glucose concentration in mM^{-1}. The point indicated by the intersection of the two dashed lines is that concentration of D-glucose at which exchange transport is unaffected by MPL. Each point is the average of five experiments. The curves drawn through the points were calculated by the method of least squares.

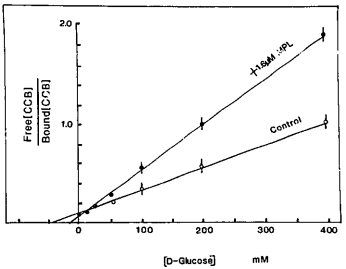

Fig. 6. Plot of the ability of D-glucose to inhibit cytochalasin B binding to the human erythrocyte sugar transporter in the presence (●) and absence (○) of monopalmitoyl lecithin (1.6 μM; MPL $1.5 \cdot 10^{-17}$ mol MPL/red blood cell). Ordinate: free/bound [cytochalasin B]. Abscissa: D-glucose concentration. The intercept on the abscissa represents $K_{i(app)}$ for D-glucose inhibition of cytochalasin B binding to ghosts. Total [cytochalasin B] was 500 nM. Number of ghosts per assay, $6 \cdot 10^8$. Temperature, 4°C. The curves drawn through the points were calculated by the method of least squares. Each point was taken in duplicate.

measurable above sugar concentrations of 25 mM D-glucose and increases, with increasing sugar levels. Below 25 mM D-glucose, exchange exit is slightly increased. Lineweaver-Burk analysis of the concentration dependence of glucose exchange exit (Fig. 5) demonstrates that under these conditions MPL reduces $K_{m(app)}$ for exit from 63.6 ± 3.9 mM in control cells to 13.3 ± 0.9 mM in MPL-treated cells. V_{max} for exit is reduced from 16.7 ± 0.7 mmol/l cell water per min in control cells to 6.8 ± 0.1 mmol/l cell water per min in treated cells (Fig. 5).

MPL ($1.5 \cdot 10^{-17}$ mols/red cell) enhances the ability of D-glucose to inhibit [^{3}H]cytochalasin B binding to the cytochalasin-D-insensitive cytochalasin B binding sites of the red cell membrane (Fig. 6). $K_{i(app)}$ for glucose inhibition of cytochalasin B binding falls from 43 ± 1 mM in control ghosts to 18.3 ± 1.7 mM in MPL-treated ghosts. Control experiments indicate that MPL is without effect on $K_{d(app)}$ for cytochalasin B binding to and the number of cytochalasin B binding sites in red-cell ghosts in the absence of D-glucose.

The effects of various lysolipids on exchange D-glucose transport are shown in Figs. 7 and 8. Fig. 7 lists exchange D-glucose (100 mM) transport rates for a series of red blood cells under control conditions (absence of lysolipid and $HgCl_2$), under conditions where carrier-mediated exchange is inhibited by $HgCl_2$ (no lysolipid), and for cells exposed to a range of either monopalmitoyl (C_{16}), monostearoyl (C_{18}), or monooleoyl (C_{18} *cis* 9-10 unsaturate) lecithin (phosphatidylcholine) levels. Experiments were performed under conditions where red blood cells were exposed to lysolipids for 15 min. It should be noted that, for all lysolipids examined, lysolipid association with the red-cell membrane was reproducibly maximal at 10–20 min incubation at 37°C, then declines slightly thereafter to achieve equilibrium association within 40–60 min (Fig. 2). It might be noted, that, while we observed increased inhibition of transport as lysolipid concentrations went

$[HgCl_2]$ mM	0	0	0	0	0	0	0.5
[lysolipid] μM	0	0.16	1.6	2.4	2.8	3.2	0
moles lipid/RBC x 10^{18}	0	1.5	15	22	26	30	0
mol lipid/mol RBC lipid x 10^2	0	0.12	1.2	1.8	2.2	2.4	0
molecules lipid/transporter	0	5.8	58	88	104	118	0

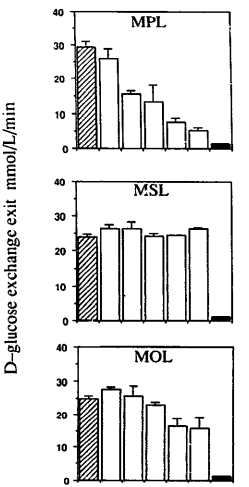

Fig. 7. Exchange D-glucose (100 mM) under conditions where carrier mediated exchange is inhibited by $HgCl_2$ (absence of lysolipid, filled bars); transport rates for a series of red blood cells under control conditions (absence of lysolipid, dashed bars) and for cells exposed to a range of concentrations of either monopalmitoyl lecithin (MPL), monostearoyl lecithin (MSL), or monooleoyl lecithin (MOL) levels. Lysolipid levels are expressed as: (1) molarity of lysolipid; (2) mol lysolipid per red blood cell; (3) mol lysolipid/mol total red blood cell membrane lipid; and (4) number of lysolipid molecules per number of transporter molecules.

[$HgCl_2$] mM	0	0	0	0	0	0	0.5
[lysolipid] μM	0	0.16	1.6	2.4	2.8	3.2	0
moles lipid/RBC x 10^{18}	0	1.5	15	22	26	30	0
mol lipid/mol RBC lipid x 10^2	0	0.12	1.2	1.8	2.2	2.4	0
molecules lipid/transporter	0	5.8	58	88	104	118	0

[$HgCl_2$] mM	0	0	0	0	0	0	0.5
[lysolipid] μM	0	0.16	1.6	2.4	2.8	3.2	0
moles lipid/RBC x 10^{18}	0	1.5	15	22	26	30	0
mol lipid/mol RBC lipid x 10^2	0	0.12	1.2	1.8	2.2	2.4	0
molecules lipid/transporter	0	5.8	58	88	104	118	0

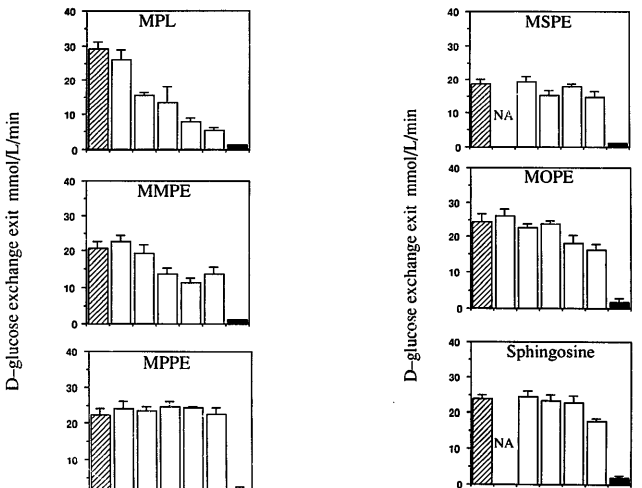

Fig. 8. Exchange D-glucose (100 mM) transport rates for a series of red blood cells under control conditions (absence of lysolipid, dashed bars); under conditions where carrier-mediated exchange is inhibited by $HgCl_2$ (absence of lysolipid, filled bars); and for cells exposed to a range of concentrations of several classes of phospholipids: monopalmitoyl lecithin (MPL), monomyristoylphosphatidylethanolamine (MMPE), monopalmitoylphosphatidylethanolamine (MPPE), monostearoylphosphatidylethanolamine (MSPE), monooleoyl phosphatidylethanolamine, and sphingosine. Lysolipid levels are expressed as: (1) molarity of lysolipid; (2) mol lysolipid per red blood cell; (3) mol lysolipid/mol total red blood cell membrane lipid; and (4) number of lysolipid molecules per number of transporter molecules.

above the CMC, we saw no qualitative difference in effect. Fig. 8 compares the modulation of protein-mediated exchange D-glucose (100 mM) transport rates of various concentrations of several classes of lysolipid: monopalmitoyl lecithin and monomyristoyl, monopalmityol, monostearoyl, monooleoyl phosphatidylethanolamine and the lysolipid analogue, sphingosine.

Discussion

Lysolipids represent a small but significant percentage of red blood cell membrane lipids. The ratio of red blood cell polar lipids (phospholipids + glycolipids) to neutral lipids (cholesterol) is approx. 1.0 : 0.9 [23]. Of the phospholipids, lysolipids comprise several percent [24]. Lysophosphatidylcholine is the most abundant of the membrane lysolipids, being normally present as 1–3% of total membrane phospholipid [10]. Approximately half of the membrane lysophosphatidylcholine is monopalmitoylphosphatidylcholine (Ref. 10; our unpublished data), making it the most abundant of membrane lysolipid species. Other lysolipids found in significant amounts are lysophosphatidylethanolamine, lyso-

phosphatidylserine, and the lyso analogue, sphingosine (our unpublished data).

This study demonstrates in vitro how very small amounts of lysolipids presented exogenously to red blood cells strongly modulate red-blood-cell sugar transport. Our work demonstrates that these lysophospholipids reversibly associate with the erythrocyte plasma membrane and, in doing so, reversibly inhibit protein-mediated glucose transport. This inhibition displays a preferential sensitivity to different lysolipids – the most potent inhibitor of transport observed being the most naturally abundant, monopalmitoyl lecithin (MPL). Increasing the presence in the red cell membrane of MPL by 1.2% (of total membrane lipid) reduces sugar transport by 50% and an increase of 2.4% MPL reduces transport by 85% (Fig. 7). That such small changes in a specific membrane lysolipid species can have such a profound effect on transport activity suggests a strong specificity in lysolipid/target protein interactions.

While there is no net synthesis or catabolism of the major lipids in mature red blood cells [10], mechanisms exist whereby membrane lysolipid concentrations can be altered in vivo. Among these mechanisms are: (a) the rapid exchange of lysolipids between membranes and plasma; (b) the deacylation of a membrane diacylphospholipid; (c) the acylation of membrane lysolipids into diacylphospholipids utilizing fatty acids, ATP, and coenzyme A; or (d) the condensation of two molecules of lysophosphatidylcholine into phosphatidylcholine and glycerophosphatidylcholine [25]. Any of these mechanisms could be a means of altering red-blood cell sugar transport rates by altering membrane levels of lysophosphatidylcholine.

The effects of MPL on sugar transport were characterized in some detail. In the presence of MPL, $K_{i(app)}$ for MPL-inhibition of transport falls with increasing D-glucose concentration. The presence of MPL results in a reduction in $K_{m(app)}$ and V_{max} for exchange D-glucose transport. These inhibition characteristics are consistent with a hyperbolic mixed-type inhibition of transport [26]. With this type of inhibition, the apparent affinity of carrier for substrate is increased by the presence of inhibitor and vice-versa. In addition, the catalytic activity of the carrier · substrate · inhibitor complex is lower than that of the inhibitor-free complex. Additional estimations of $K_{m(app)}$ and V_{max} for exchange D-glucose transport over a range of MPL levels would be required to confirm this interpretation of the transport data.

A number of mechanisms could give rise to MPL inhibition of glucose transport. These include: (1) a nonspecific, detergent-like or membrane bilayer perturbing or distorting (e.g., red cell shape changes – 'invaginations' or 'crenations') action of lysophosphatidylcholine on red-cell membranes; (2) an alteration in the packing of the lipids surrounding the transporter; and (3) the direct interaction of MPL (or a metabolite of MPL) with the glucose transport protein.

The first mechanism seems unlikely in view of the specificity of transport inhibition and the low amounts of lysolipids required to inhibit sugar transport activity. In addition, Fujii et al. [27] demonstrated that, while treatment of intact red cells with phospholipase A_2 induced invaginations of the cell membrane, even at 21% hydrolysis of total membrane phosphatidylcholine there was negligible change in sugar transport. An interesting recent study opens up a variation of this mechanism for consideration. Golan et al. [28] found in studies on red cell ghosts that lysophosphatidylcholine can induce distinct types of membrane region or domain called wrinkles and patches. Membrane proteins and lipids were found to be immobilized in these domains. It is possible that the sugar transport molecule is effected somehow by the formation of patches and wrinkles, either being incorporated into them or else having their normal lipid environment changed by patch or wrinkle formation. This observation is similar to our second postulated mechanism of MPL action, a perturbation of the packing of the lipids immediately surrounding the transporter. Although approx. 58 molecules of MPL per transport molecule are required for a significant MPL effect, much of this MPL may partition into regions of the membrane lacking the sugar transporter or else bind to other membrane proteins.

A comparison of our findings with those of Fujii et al. [27] would suggest that several pools of lysolipid exist in the membrane. Lysolipids entering the membrane from exogenous sources do not effect sugar transport in the same manner as lysolipids formed in the membrane from existing membrane lipids by the action of bee-venom phospholipase A_2. In the study by Fujii et al., PLA_2 treatment of red cells reduced both $K_{m(app)}$ and V_{max} for glucose uptake at 25°C. This result is entirely consistent with our observation of MPL reduction of $K_{m(app)}$ and V_{max} for glucose exchange at 4°C. Fujii et al. found, however, that exposure of PLA_2 treated cells to BSA reversed the effect of PLA_2 treatment. Although BSA is known to bind lysolipids strongly [29], albumin at the low concentrations used by Fujii et al. was reported to remove almost all the fatty acids and only some of the phosphatidylcholine produced in the membrane [27]. Therefore, the investigators believe that the fatty acids released into the external hemileaflet upon phospholipid hydrolysis are responsible for transport inhibition by exogenous PLA_2, not the newly formed lysophospholipids. Thus, while lysophospholipids formed from endogenous membrane lipids by an extrinsic PLA_2 appear not to alter sugar transport, exogenous lysolipids entering the membrane are able to inhibit sugar transport. It is possible, however, that red-cell membrane phospholipid hydrolysis by exoge-

nous PLA_2 might not generate appropriate inhibitory, lysolipid species in sufficient quantities to inhibit transport. The identity of lysolipid species generated by the treatment of red cells with PLA_2 was not reported in the study by Fujii et al.

A consistent observation in our studies was the biphasic inhibition of exchange sugar exist with time (Fig. 2). Maximum inhibition of exit is observed at 15 min incubation with MPL thereafter, some recovery occurs – inhibition reaching a new steady-state within 60 min. There are several possible explanations for this. The phenomenon may reflect hysteresis between the equilibria for lysophosphatide adsorption and desorption between cell membrane and solution [24]. The biphasic inhibition is also consistent with the major site of MPL action being the outer hemileaflet of the membrane bilayer. Initially, MPL partition into the outer hemileaflet (99% of the MPL is bound within 15 min). As flip-flop proceeds, MPL will become more evenly distributed between each hemileaflet of the bilayer. Alternatively, metabolic depletion of membrane associated MPL might offer an explanation for biphasic inhibition of transport. Another possibility could be argued from findings by Golan et al. [28] on lysolipid-induced membrane patch formation, e.g., that the time-course of sugar transport inhibition by lysolipids reflects the kinetics of lysolipid participation in patch formation.

As discussed, the action of MPL on sugar transport could reflect local perturbations in bilayer structure surrounding the carrier or result from a direct interaction of MPL with the carrier. Conventional binding studies using radiolabelled MPL and purified glucose transporter are not useful owing to the complex solubility of lysolipids in lipid bilayers. Activation of phenylalanine hydroxylase from rat liver by lysophosphatidylcholine has been correlated with a lysophosphatidylcholine-induced modification of the enzyme's conformation [3]. The mode of interaction of MPL with the erythrocyte glucose transporter remains to be established.

The sensitivity of a membrane transport system to low amounts of a specific membrane lipid transport system underscores the importance of bilayer lipid composition in membrane enzyme function. Studies in reconstituted systems on the effect of lysophosphatidylcholine and cholesterol on microsomal glucose-6-phosphatase illustrate the subtlety of how a specific lipid can effect a membrane activity. Chauhan et al. [2] demonstrated that the addition of cholesterol to the bilayer reverses the inhibition of glucose-6-phosphatase by lysolipid. This reversal is proposed to result from a complexing of cholesterol with lysolipid through hydrogen bonding in the hydrogen belt region of the bilayer. The preference of cholesterol for lysophosphatidylcholine over phosphatidylcholine appears to be roughly of one order of magnitude. Thus, the interesting possibility exists that the efficacy of various lysolipids in modulating sugar transport can in turn by modulated by overall bilayer cholesterol levels as well as cholesterol levels in the local environment of the sugar transport molecule.

Our studies were carried out at 0°C. Qualitatively, these studies would be expected to extrapolate to physiological temperatures. Lowe and Walmsley [30] have demonstrated that the temperature profile for equilibrium exchange transport in the red cell is a continuous function of temperature. The direct physiological relevance of lysolipid modulation of glucose transport in erythrocytes remain to be established. Conditions known to stimulate the endogenous PLA_2 activity of cells (e.g., raised Ca^{2+} [21–33] inhibit glucose transport in the red cell [34,35]. The glucose carriers of rat adipocytes, rat brain cells, Hep G2 cells and human erythrocytes share significant functional, structural, and immunological homologies [36]. It is not unreasonable to speculate that erythrocyte sugar transport system or other membrane activities may be susceptible to modulation by specific lysolipids.

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