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Effect of Cholesterol and Dipalmitoyl Phosphatidylcholine Enrichment on the Kinetics of Na-Li Exchange of Human Erythrocytes

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Summary. The effects of cholesterol loading and depletion and of a 10% replacement of native phosphatidylcholine by dipalmitoyl phosphatidylcholine (di 16 : 0-PC) on kinetic properties of human red cell Na-Li exchange have been studied.

Compared to control erythrocytes (cholesterol/phospholipid ratio (C/P = 0.8–0.9)), V_{max} of phloretin-sensitive Li uptake and of Li efflux stimulated by extracellular Na (Na_o) were reduced by 15–30% in cholesterol-loaded red cells (C/P = 1.05–1.33). The apparent K_m values for external Li (Li_o) and for internal Li (Li_i) were decreased by about one-third in these cells. Cholesterol depletion (C/P = 0.7) exerted opposite effects on the kinetics of Na_o-dependent Li efflux. On augmenting C/P from 0.66 to 1.0, V_{max} of Na_o-dependent Li efflux was reduced by about 30%; increasing C/P above 1.0 caused no further lowering of V_{max}. Li leakage rates monotonically decreased over the whole range of C/P ratios examined (0.66–1.3). This indicates that Na-Li exchange and Li leak are differentially affected by cholesterol.

Incorporation of di 16 : 0-PC (replacement of 3% of total red cell phospholipids) caused similar kinetic alterations of Na-Li exchange as a rise in membrane cholesterol by 20–50%. Notably, selective incorporation of di 16 : 0-PC into the outer monolayer increased both intra- and extracellular Li binding affinities of Na-Li exchange and lowered its maximum velocity. Thus, both di 16 : 0-PC enrichment and cholesterol loading exerted an uncompetitive type of transport inhibition. The results are in agreement with the hypothesis that the kinetic alterations of red cell Na-Li exchange seen in a subgroup of essential hypertensive patients could be due to subtle changes in the molecular species composition of individual phospholipids.

Key Words transport · membrane · lipid-protein interactions · essential hypertension · inner and outer monolayer

Introduction

It has repeatedly been shown that some kinetic and physico-chemical properties of red cell Na-Li exchange are altered in a subgroup of essential hypertensive patients: (*i*) V_{max} is increased, and the apparent affinity for intracellular Na is decreased [2, 6, 12]. (*ii*) Arrhenius plots of Li efflux mediated by the Na-Li exchange indicate a drop in the break temperature from about 30°C in normotensives to around 20°C in essential hypertensives [22]. It has been hypothesized that changes in the lipid environment—possibly induced by alterations of plasma lipids—could account for these alterations [9, 21]. It has previously been shown that limited replacement of native red cell phosphatidylcholine (PC)[1] by different molecular species of PC can cause considerable changes in Na-Li exchange rate and that these alterations depend on the distribution of the PC species over the two leaflets of the membrane [10].

The experiments of the present study were designed to extend these investigations by studying the effects of cholesterol enrichment and depletion as well as of partial replacement of native phosphatidylcholine by a disaturated molecular species of this phospholipid (di 16 : 0-PC) on the kinetic parameters of red cell Na-Li exchange. In order to get a rather complete picture of potential effects of the lipid modifications on Na-Li exchange, intra- and extracellular Li binding affinities and maximum Li transport rates were assessed. The kinetic parameters were derived from measurements of phloretin-sensitive Li uptake determined as a function of extracellular Li in erythrocytes with normal or elevated Na contents, as well as from determinations of Na_o-dependent Li efflux assessed as a function of intracellular Li.

Attention was focused on the role of localization of newly inserted lipid molecules for potential effects on extra- and intracellular cation binding affinities. Di 16 : 0-PC inserted into the membrane is nearly

[1] *Abbreviations*: PC, phosphatidylcholine; di 16 : 0-PC, dipalmitoyl phosphatidylcholine; C/P ratio, cholesterol/phospholipid ratio; subscripts $_o$ and $_i$, extracellular and intracellular; PE, phosphatidylethanolamine; Sph, sphingomyelin; PS, phosphatidylserine; PI, phosphatidylinositol; V_{max}, apparent maximum velocity; K_m, apparent dissociation constant; and MCHC, mean cellular hemoglobin concentration.

exclusively located in the outer monolayer under the experimental conditions applied [10], whereas incorporation of cholesterol—due to rapid transbilayer movement of the sterol [19]—will probably result in a redistribution over both leaflets.

It has indeed been shown earlier that another red cell Na transport system, the Na, K pump, appears to be rather selectively sensitive to modifications of inner monolayer fluidity [14, 25], whereas changes in outer monolayer fluidity were without effect on the kinetic parameters of this Na transport system.

Materials and Methods

Erythrocytes

Fresh venous blood (anticoagulated by 5 IU heparin/ml) was obtained from normotensive donors. After careful removal of the buffy coat, the cells were washed four times with 10 vol of a solution containing (in mM): 145 NaCl, 5 KCl, 5 glycylglycine, and 5 glucose (pH 7.4 at 37°C), referred throughout as Na buffer.

Cholesterol Loading and Depletion

Vesicles with different ratios of cholesterol and di 16:0-PC were prepared in order to enrich, deplete or maintain red cell cholesterol content. Usually, 30 mg of di 16:0-PC and 0–60 mg of cholesterol (both obtained from Sigma, St. Louis, MO) were suspended in 8 ml of a buffer containing (in mM): 145 NaCl, 35 sucrose, 5 KCl, 10 glucose, 10 Tris-3-(N-morpholino)-propanesulphonic acid (Tris-Mops buffer), 1 $MgCl_2$, 1 phosphoric acid, and 0.1 EGTA (pH 7.4 at 37°C), referred throughout as Na-sucrose buffer. The mixture was sonicated for three 10-min periods under N_2 at 4°C and 60 W with 2-min breaks in between using a Branson sonifier. Thereafter, the suspension was centrifuged for 30 min at 21,000 × *g* and 4°C in N_2 filled tubes in order to remove large vesicles and titanium particles. Packed erythrocytes (2.4 ml) were incubated with 7 ml of vesicle suspension and 14 ml of the Na-sucrose buffer containing 2000 IU penicillin/ml for 12–16 hr at 37°C under N_2. One hr before the end of the incubation, 2 mM adenine and 10 mM inosine were added to the media to restore red cell ATP levels.

After the end of the long-term incubation, red cell Na and K levels and MCHC values (to estimate red cell volume) were determined. Na and K contents of cholesterol-modified cells fell within a normal range (6.2–8.1 and 87.4–94.1 mmol/liter, respectively). MCHC values (mmol hemoglobin/liter cells, by tetramer) were 5.23 (±0.11) in cholesterol-loaded cells (n = 9, C/P = 1.07), 5.22 (±0.13) in cholesterol-depleted cells (n = 8, C/P = 0.70) as compared to 5.18 (±0.14) in cholesterol-maintained cells (n = 8, C/P = 0.86, means ± SD). Hemolysis was in all cases less than 1%. For every Li flux experiment in cholesterol-modified erythrocytes (*see below*) a sample with cholesterol-maintained cells was run in parallel which served as control. In two experiments, V_{max} values of Na_o-dependent Li efflux of red cells incubated for 14 hr with vesicles containing cholesterol and di 16:0-PC in a molar ratio of 0.85:1.0 were compared with red cells incubated for the same time period in the Na-sucrose buffer without vesicles. The V_{max} values differed by less than 7% (0.30 and 0.32 mmol (liter cells · hr^{-1}) (vesicle treated) *vs.* 0.28 and 0.32 (without vesicles)).

Di 16:0-PC Enrichment

The protocol used was the same as previously described [10]. Vesicles were prepared from di 16:0-PC, cholesterol and egg phosphatidic acid (all from Sigma) in a molar ratio of 1:0.8:0.1, and in some experiments, trace amounts of dipalmitoylphosphatidyl-[^{14}C]-choline (Amersham, UK). The lipids were dispersed in the Na-buffer and sonicated five times for 40 sec at 4°C under N_2. Subsequently, the suspension was centrifuged for 30 min at 4°C and 12,000 × *g*. Eighty-five percent of the supernatant was used as vesicle suspension. The phosphatidylcholine specific-transfer protein (PC-T_p) was purified and prepared as described in [18, 28]. Washed red cells were incubated for 2 hr at 37°C and a hematrocrit of 20% with the donor vesicles in the absence (control cells) or presence of the PC-T_p (final concentration about 3 μM) under N_2. After the end of the incubation, the erythrocytes were washed four times in buffer containing (in mM): 75 $MgCl_2$, 90 sucrose, 5 glucose, and 10 Tris-Mops (pH 7.4 at room temperature), referred to as Mg-sucrose buffer. Replacement of native PC by di 16:0-PC was estimated by measuring the radioactivity of ^{14}C-labeled di 16:0-PC present in membrane lipid extracts using the calculations given in [26]. In four separate experiments, 9.6 ± 2.6% (means ± SD) of native PC were calculated to be replaced by di 16:0-PC after the 2 hr incubation in the presence of PC-T_p- and di 16:0-PC-containing vesicles. In two experiments, V_{max} values of Na-Li exchange were compared in cells previously treated for 2 hr with vesicles containing cholesterol and di 16:0-PC in a molar ratio of 0.85:1 (absence of the PC-T_p) and in red cells incubated for 2 hr in the Na-buffer without vesicles. The V_{max} values differed by less than 6% (Na_o-dependent Li efflux: 0.31 (vesicle treated) *vs.* 0.29 mmol (liter cells · hr)$^{-1}$ (without vesicles); Li influx into Na-loaded cells: 0.56 (vesicle treated) *vs.* 0.54 mmol (liter cells · hr)$^{-1}$ (without vesicles).

Analysis of Membrane Lipids

Red cell membrane lipids were extracted according to Rose and Oklander [23]. For cholesterol measurements, the dry extract was taken up in ethanol and cholesterol was quantitated with a Boehringer (Mannheim, FRG) kit by the cholesterol oxidase method. The phospholipid content of chloroform:methanol (2:1) extracts was quantitated by phosphate determinations according to [13]. Phospholipids were separated on Merck DC Fertigplatten (Kieselgel 60) by using the developing systems according to Broekhuyse [1].

Phloretin-sensitive Li Uptake of Normal Na Erythrocytes

Packed red cells (0.2 ml) which had previously been washed five times with the Mg-sucrose buffer, were suspended in 10 ml of prewarmed (37°C) Mg-sucrose buffer containing five different concentrations of LiCl between 0.5 and 5 mM and 0.2 mM ouabain. After 1 hr incubation in the presence or absence of phloretin (0.2 mM), red cells were washed three times in ice-cold isosmotic choline chloride. Red cells were hemolyzed with 6% *n*-butanol in H_2O (vol/vol), and the Li concentrations in the supernatants were

measured by atomic absorption. The Li content of the erythrocytes was normalized to 5.2 mmol of hemoglobin, the mean hemoglobin content of 1 liter of normal erythrocytes.

Phloretin-Sensitive Li Uptake of Na-Loaded Erythrocytes

Red cells were loaded with Na by using a method described by Garay [11]. Three ml of washed erythrocytes were incubated for 90 min at 37°C with 75 ml of a 100 mM Na_2HPO_4-75 mM sucrose solution, the medium being renewed after 30 and 60 min. Thereafter, the red cells were incubated for 80 min in 27 ml of a buffer containing (in mM): 150 NaCl, 10 glucose, 10 inosine, 10 Tris-Mops buffer, and 1 phosphoric acid (pH 7.4 at 37°C), the medium being renewed after 40 min. After the loading procedure, red cell Na contents ranged between 33.0 and 42.9 mmol/liter, and MCHC values were 5.28 ± 0.15 mmol hemoglobin/liter cells (n = 13). The cells were subsequently washed five times with the Mg-sucrose buffer. Packed erythrocytes (0.2 ml) were added to 10 ml of prewarmed Mg-sucrose buffer containing (in mM): 0.2 ouabain and 1, 2, 5, 10 and 20 LiCl, respectively. The osmolarity in the media was kept at 300–305 mOsm/Kg H_2O by appropriate reductions of $MgCl_2$. After a 1 hr incubation at 37°C in the presence or absence of 0.2 mM phloretin, the cells were washed and analyzed for their Li contents as described above.

Na_o Dependent Li Efflux

Washed erythrocytes were loaded with Li by incubation of the cells with 140 mM LiCl, 10 mM glucose, 10 mM Tris-Mops buffer (pH 7.45 at 37°C) for different time periods between 2 and 150 min at 10% hct and 37°C. Li-loaded red cells were subsequently washed five times with Mg-sucrose buffer, 0.2 mM ouabain being present during the last wash. Aliquots of the cells were analyzed for their Na and Li contents (0.2–6 mmol Li/liter, 2.5–6 mmol Na/liter). Packed red cells (0.2 ml) were suspended in 1.75 ml of either Mg-sucrose buffer or a 145 mM NaCl, 5 mM glucose, and 10 mM Tris-Mops buffer (pH 7.45 at 37°C, 0.2 mM ouabain). After 1 hr incubation at 37°C, the cells were washed and red cell Li contents were measured as described above. Na_o-dependent Li efflux was estimated from the differences in Li content of the cells incubated in the two media.

Statistical Analysis

All V_{max} and apparent affinity constants for intra- and extracellular Li were calculated using the Eadie-Hofstee plot. For all curve fittings given, r-values exceed 0.96.

Statistical analysis was performed by unpaired Student's t test. P values of greater than 0.05 were considered insignificant.

Results

Incubation of red cells with vesicles of different C/P ratios resulted in either elevation, reduction or maintenance of the cholesterol content of the red cell membrane (Table 1). In red cells with normal Na content, the modifications of the cholesterol content were not accompanied by changes in the total phospholipid content of the membrane, the changes in membrane C/P ratios thus being exclusively due to differences in cholesterol content. In cells loaded with both cholesterol and Na, the phospholipid content was slightly reduced by 5% (Table 1). The overall phospholipid composition of cholesterol enriched, depleted or maintained erythrocytes was largely unaffected as compared to untreated (native) erythrocytes (Table 2).

When Na-Li exchange was assessed by measuring phloretin-sensitive Li uptake of cells with normal Na content, excess cholesterol of 38% caused a 19% decrease in V_{max} and a 37% reduction in the apparent binding constant for extracellular Li (Km_{Li_o}, upper panel of Fig. 1), i.e., an increase in the affinity for Li_o. Replacement of about 10% native PC by di 16:0-PC resulted in similar concomitant reductions of both V_{max} and apparent Km_{Li_o} by 15 and 22%, respectively (lower panel of Fig. 1).

In order to assess Li^+ uptake through the Na-Li exchange under saturating conditions for intracellular Na, red cells were loaded with Na according to [11]. In a control experiment it was found that an increase of intracellular Na from 6.95 to 38.7 mmol/liter accelerated V_{max} of phloretin-sensitve Li uptake by about 2.5-fold (from 0.20 to 0.51 mmol (liter cells · hr)$^{-1}$). In addition, the apparent Km_{Li_o} was considerably higher in Na-loaded cells (3.85 in Na-loaded *vs.* 1.35 mmol/liter in untreated erythrocytes), in accordance with results previously reported for human and sheep erythrocytes [17, 24]. In Na-loaded red cells (Fig. 2), an excess cholesterol of 31% as well as di 16:0-PC enrichment again caused a reduction in both V_{max} (of 15 and 29%, respectively) and in the Km_{Li_o} of phloretin-sensitive Li uptake (by 25 and 40%, respectively).

In order to study the effect of cholesterol and di 16:0-PC enrichment on the apparent affinity of Na-Li exchange for intracellular Li(Km_{Li_i}), red cells, in which the membrane lipid composition had been modified, were loaded with Li, and thereafter, Li efflux was registered as detailed in Materials and Methods. Excess cholesterol of 56% reduced both V_{max} and Km_{Li_i} of Na_o-dependent Li efflux by about one-third in the particular experiment shown in the middle panel of Fig. 3. Cholesterol depletion by about 8%, in contrast, caused a stimulation of V_{max} by 16%, as well as an increase of Km_{Li_i} of 14% (Fig. 3, upper panel). In di 16:0-PC-enriched cells, the V_{max} was reduced by 28% and the apparent Km_{Li_i} was decreased by 32% (lower panel in Fig. 3).

The results of similar experiments are plotted in percent against control erythrocytes in Fig. 4. In di

Table 1. Effect of cholesterol loading and depletion on cholesterol and phospholipid content of normal and Na loaded erythrocytes

Lipid modification	Cholesterol content (mmol/liter cells)	Phospholipid content (mmol PO_4/liter cells)	Cholesterol/phospholipid
Red cell Na 6–8 mmol/liter			
None (native red cells) (n = 2)	3.51	4.08	0.86
Cholesterol maintained (n = 4)	3.44 ± 0.21	4.09 ± 0.09	0.84
Cholesterol loaded (n = 5)	4.52[c] ± 0.47	4.08 ± 0.13	1.11
Cholesterol depleted (n = 5)	2.93[c] ± 0.16	4.05 ± 0.17	0.72
Red cell Na 33-43 mmol/liter			
Cholesterol maintained (n = 3)	3.21 ± 0.17	3.92 ± 0.11	0.82
Cholesterol loaded[a] (n = 3)	3.97[c] ± 0.63	3.87[b] ± 0.07	1.03

Cholesterol and phospholipid contents were measured as described in Materials and Methods.
[a] Red cells were first incubated for 14 hr with the appropriate vesicles to maintain or elevate red cell cholesterol and thereafter loaded with Na as described in Materials and Methods. Means ± SD.
[b] $P < 0.05$ (versus cholesterol loaded cells with normal Na content).
[c] $P < 0.01$ (versus cholesterol maintained).

Table 2. Effect of cholesterol loading and depletion on the phopholipid composition of human erythrocyte membrane

Lipid modification	Phospholipid composition (%)			
	PC	PE	Sph	PS/PI
None (native red cells) (C/P = 0.86, n = 3)	33.4 ± 2.5	28.8 ± 1.4	27.4 ± 2.7	10.3 ± 0.7
Cholesterol maintained (C/P = 0.85, n = 2)	32.1	28.9	28.8	10.2
Cholesterol loaded (C/P = 1.18, n = 3)	34.4 ± 3.0	25.4 ± 0.9	30.1 ± 3.4	10.1 ± 3.1
Cholesterol depleted (C/P = 0.60, n = 2)	33.5	27.4	29.3	9.7
Cholesterol maintained[a] Na loaded (C/P = 0.82, n = 2)	33.7	29.8	26.0	10.5

Red cell membrane lipids were extracted according to ref. 14 and phospholipids separated on TLC by using the developing systems in [16].
[a] Red cells were first incubated for 14h with vesicles appropriate to maintain their C/P ratio and thereafter loaded with Na as described in Methods. Mean values ± SD.

16:0-PC-enriched cells, the apparent K_m values for both intra- and extracellular Li were decreased by 20–40% and V_{max} values were reduced by 15–30% (upper panel of Fig. 4). Excess cholesterol caused similar reductions in the K_m values for both intra- and extracellular Li by 30–38%, as well as of V_{max} values that were lowered by 14–25% (lower panels of Fig. 4).

Figure 5 summarizes mean V_{max} values of Na_o-dependent Li efflux (upper curve) and of Li leakage rates (Li efflux into Mg media, lower curve) of red cells in which the C/P ratio of the membrane was varied over a range from 0.66 to 1.31. At C/P ratios between 0.66 and 1.02, the V_{max} values exhibited a rather steep decrease of about 30% with increasing cholesterol content, whereas at C/P ratios above

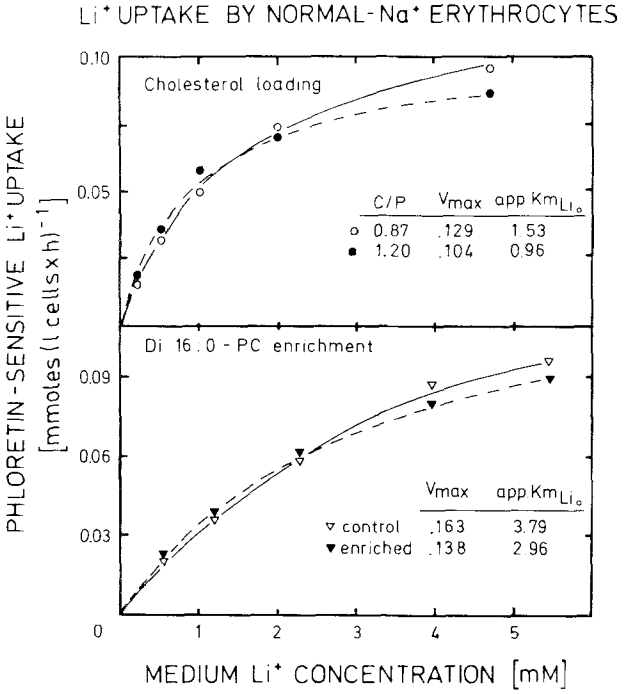

Fig. 1. Phloretin-sensitive Li uptake by cholesterol-loaded and di 16:0-PC-enriched erythrocytes (10% of total PC) with normal Na content. The Na contents as determined before the start of Li uptake were 3.71 and 3.58 mmol/liter cells (upper panel, C/P 0.87 and 1.2, respectively) and 7.13 and 7.80 mmol/liter cells (lower panel) in di 16:0-PC-enriched cells and control erythrocytes. The data shown in the upper and lower panels were derived from red cells of different donors. Data were from one of two (di 16:0-PC) or three (cholesterol) similar experiments. The curves are plotted according to the apparent kinetic parameters given in the figure

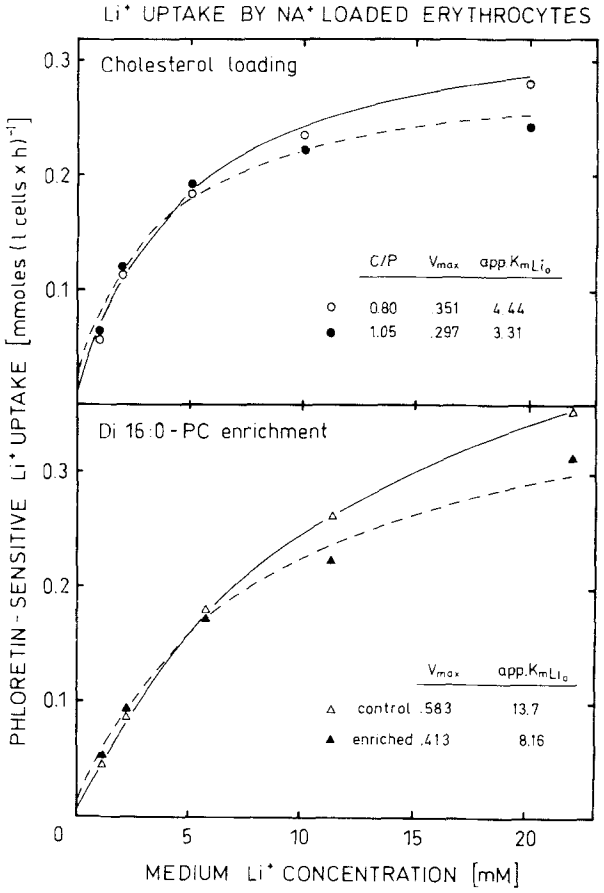

Fig. 2. Phloretin-sensitive Li uptake by cholesterol-loaded and di 16:0-PC-enriched erythrocytes (10% of total PC) loaded with Na. After modification of the lipid composition, red cells were loaded with Na as detailed in Materials and Methods. Red cell Na contents before the start of Li uptake were: 33.4 and 33.0 mmol/liter in red cells (upper panel) with C/P ratios of 0.8 and 1.05, respectively, and 42.3 and 42.9 mmol/liter cells (lower panel) in control and di 16:0-PC-enriched erythrocytes. The data shown in the upper and lower panels were obtained on erythrocytes from different donors. Data were from one of two similar experiments. The curves are plotted according to the kinetic parameters given in the figure

1.02, no further reduction in V_{max} values was observed. As can be deduced from the lower curve of Fig. 5, Li leakage rates decreased monotonically with rising membrane cholesterol content. In di 16:0-PC-enriched erythrocytes, no changes in Li leakage rates (Li efflux into Mg media) were observed compared to control erythrocytes (*data not shown*).

Discussion

Incubation of red cells with vesicles of different C/P ratios resulted in a rather selective modification of red cell cholesterol content without affecting either the total phospholipid content or the overall phospholipid composition of the red cell membrane (Tables 1 and 2) in accordance with previous results [4]. In the case of di 16:0-PC enrichment, changes in either the content or in the fatty acid composition of other phospholipids (apart from PC) are unlikely, since the PC-T_p applied to modify the membrane phospholipid composition mediates a genuine and selective exchange of native PC molecules with the di 16:0-PC molecules present in the vesicles [7, 26]. Furthermore, control experiments showed that incubation of red cells with vesicles in the absence of transfer protein for up to 14 hr did not alter V_{max} values of Li fluxes when compared with erythrocytes incubated in media without vesicles (*see* Materials and Methods). This indicates that vesicle treatment by itself did not induce changes in kinetic properties of Na-Li exchange. Accordingly, red cells treated with vesicles in order to maintain their C/P

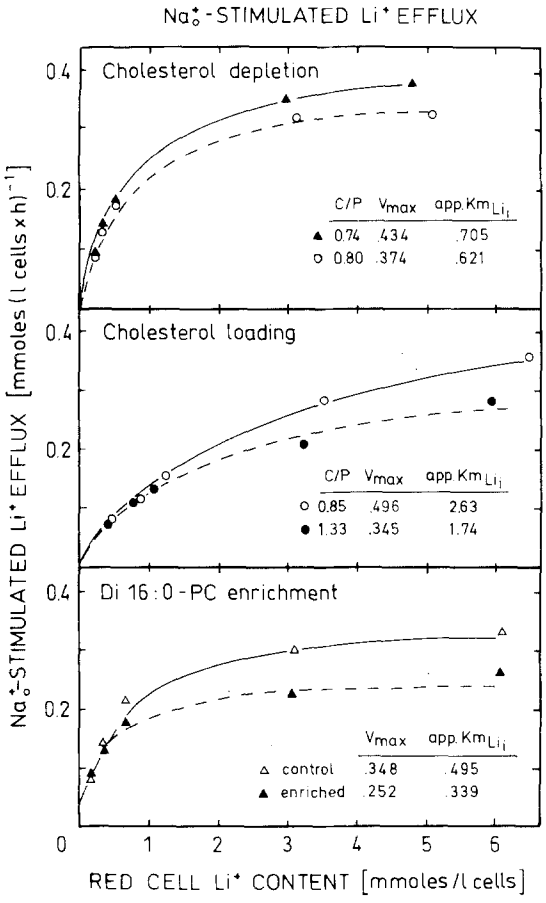

Fig. 3. Li efflux stimulated by extracellular Na in cholesterol-loaded, cholesterol-depleted and di 16:0-PC-enriched erythrocytes. After modification of the lipid composition, red cells were loaded with different concentrations of Li, and thereafter, Li efflux into either Na or Mg media was started. Red cell Na contents in Li-loaded cells ranged from 2.57 to 5.94 mmol/liter cells. The data shown in the upper, middle and lower panels were derived from red cells of different donors. Data were from one of three similar experiments. The curves are plotted according to the kinetic parameters given in the figure

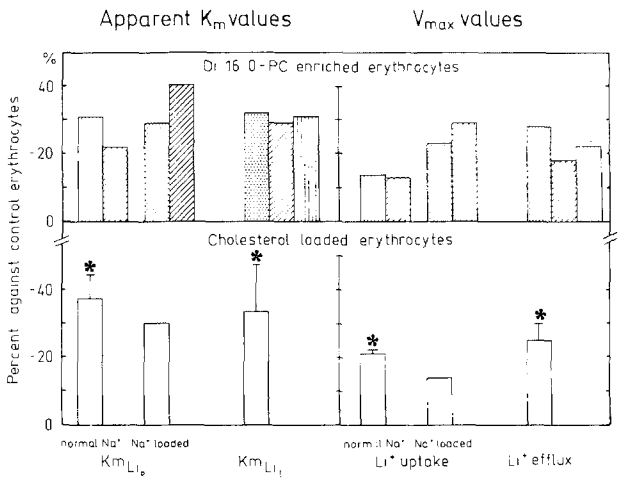

Fig. 4. Apparent K_m and V_{max} values of phloretin-sensitive Li uptake (normal Na and Na-loaded erythrocytes) and Na_o-dependent Li efflux in cholesterol-loaded and di 16:0-PC-enriched erythrocytes. Different columns for di 16:0-PC-enriched cells (10% of total PC) indicate different donors. Data on cholesterol-loaded erythrocytes were obtained on three different donors where indicated by bars (means ± SD), otherwise on two donors. The C/P ratios of cholesterol-loaded red cells were: 1.28 ± 0.09 (Li uptake normal Na red cells), 1.06 (Li uptake Na-loaded erythrocytes) and 1.33 ± 0.05 (Na_o-dependent Li efflux, means ± SD). *$P < 0.05$ against control erythrocytes (Student's paired t test)

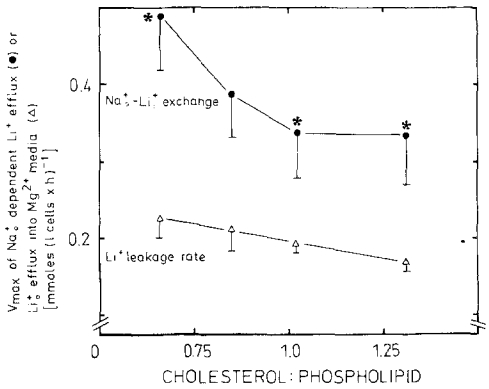

Fig. 5. V_{max} values of Na_o-dependent Li efflux and Li efflux into Mg media (Li leakage rate) as a function of the C/P ratio of the membrane. After modification of the cholesterol content of the membrane, red cells were loaded with 4.31–6.11 mmol Li/liter cells, and subsequently, Li efflux into Mg media or into Na media was registered. Data were obtained on red cells of five donors. Bars indicate means ± SD. *$P < 0.05$ against control erythrocytes (C/P = 0.85) (Student's paired t test)

ratio of about 0.85 could be safely used as control cells for the Li flux experiments with cells of modified cholesterol and di 16:0-PC contents.

Both excess cholesterol and di 16:0-PC enrichment caused a reduction in V_{max} and an increase in the apparent affinity for extracellular Li of Li uptake through the Na-Li exchange (Figs. 1 and 2). In terms of enzyme kinetics, membrane cholesterol and outer monolayer di 16:0-PC appear to act as uncompetitive inhibitors by interacting preferentially with the cation-loaded exchanger, thereby producing proportional reductions in apparent V_{max} and K_m values. Such concomitant alterations of the kinetic parameters were seen in cells with normal Na content, as well as in cells loaded with Na to saturate the inner binding siters of Na-Li exchange.

A similar kinetic pattern as for Na_i-dependent Li uptake was also seen for Na_o-dependent Li efflux in cholesterol-loaded erythrocytes, e.g., a reduction in V_{max} as well as an increase in the apparent affinity for intracellular Li. Cholesterol depletion exerted

opposite alterations (Figs. 3 and 4). Similar effects of cholesterol depletion and enrichment have already been observed for two other red cell Na transport systems, namely the Na, K pump as well as the Na, K cotransport system [15, 27].

Replacement of 10% of endogenous PC by di 16:0-PC reduced the apparent affinities for intra- and extracellular Li and the V_{max} values of inward and outward Na^+-Li^+ exchange, similar to the kinetic changes seen subsequent to cholesterol loading (Fig. 4). Thus, replacement of less than 2% of total red cell lipids by di 16:0-PC can induce comparable changes in kinetic parameters of Na-Li exchange as a 9–23% increase in total membrane lipids brought about by a 20–50% rise in membrane cholesterol (Figs. 4 and 5, assuming a red cell C/P ratio of 0.85 in native cells).

These findings appear to be partly at variance with previously observed accelerations of Na-Li exchange rate [10] measured at 2 mM Li_o in red cells enriched with about 10% di 16:0-PC (as in the present study). However, due to the concerted reduction of apparent K_m and V_{max} values of Li uptake, the transport activity of di 16:0-PC-enriched cells—as compared to control cells—is higher below the apparent Km_{Li_o} and reduced above this value (Fig. 1). Accordingly, the acceleration of Na-Li exchange rate previously reported could be explained, e.g., by higher apparent Km_{Li_o} values in red cells of the donors studied.

Since di 16:0-PC is nearly exclusively present in the outer monolayer under the experimental conditions employed [10], the present data indicate that the change in outer monolayer PC fatty acid composition brought about by incorporation of the disaturated species induces modifications of both extra- and intracellular cation binding affinities of the Na-Li exchange system.

In this respect Na-Li exchange probably differs from the Na, K pump, since incorporation of agents known to fluidize predominantly the outer leaflet of the membrane such as anionic local anesthetics did not affect the apparent affinities for extracellular K and of intracellular Na of the Na, K pump [14] or the activity of the Na, K-ATPase [25]. In contrast, cationic local anesthetics, which predominantly accumulate in the inner leaflet of the membrane, were found to increase the apparent affinity constant for intracellular Na and to increase V_{max} of the Na^+, K^+ pump [14]. Possibly, the "active center" of the putative Na, Li exchanger, which may be sensitive to its lipidic environment, is located either in the middle or in the outer half of the bilayer, in contrast to the Na, K pump whose "active center" has been suggested to be present in the inner monolayer [25].

It has been shown that cholesterol loading monotonically decreases the fluidity of the red cell membrane between C/P ratios of 0.5 and 2.0 [5]. However, the data in Fig. 5 indicate, that above a C/P ratio of 1.0, V_{max} of Na_o-dependent Li efflux is not further reduced by an increase in cholesterol content compared to a C/P ratio of about 1.0. In addition, replacement of about 10% endogenous PC by di 16:0-PC (i.e., of about 3% of total phospholipids) induces changes in V_{max} of Li uptake similar in magnitude to those seen upon augmenting the cholesterol content of the membrane by about 30% (Figs. 1 and 4); yet the increase in membrane viscosity due to even higher enrichment of di 16:0-PC is small (less than 10%, ref. [3]) compared to the 40% rise in membrane viscosity seen upon increasing membrane C/P ratio from 0.85 to 1.2 [5]. It is thus unlikely, that changes in membrane viscosity can offer an explanation for the effects of both the sterol and the phospholipid on the kinetics of Na-Li exchange. The linear decrease in Li leak (which probably reflects a nonprotein-mediated process) with rising cholesterol is consistent with earlier studies showing that ion permeabilities through human erythrocytes are diminished by incorporation of cholesterol (reviewed in ref. [8]).

Whatever the mechanism, it appears obvious from the above results that both Na-Li exchange and Li leakage rates are reduced by excess cholesterol. This observation is partly inconsistent with a previous study in which Li efflux through the Na-Li exchanger was found to be unchanged and Li leak diminished subsequent to incorporation of cholesterol hemisuccinate into the red cell membrane [20]. However, cholesterol hemisuccinate is a more hydrophilic molecule than cholesterol, and it cannot be assumed that it will fully mimick the effects of cholesterol.

It is well established that in red cells of a subgroup of essential hypertensive patients, the apparent affinity for intracellular Na (or Li) is decreased while V_{max} of Na_o-dependent Li efflux is increased [6, 12]. A similar pattern of kinetic changes is observed in the present study upon cholesterol depletion (Fig. 3). However, it appears unlikely that the cholesterol content of the red cell membrane is reduced in essential hypertensive patients, since the C/P ratio of erythrocytes from essential hypertensive patients has been reported to be normal [16]. On the other hand, the clearly established acceleration of red cell Na-Li exchange seen in a subgroup of essential hypertensive patients, as well as in a variety of other diseases (summarized in ref. [19]), could be due to subtle changes in the molecular species composition of single membrane phospholipids. Thereby, alterations in the kinetic parameters of the transport system might be induced, which are

similar and opposite to those observed in cholesterol-depleted and di 16:0-PC-enriched (or cholesterol-loaded) erythrocytes, respectively. In addition, a normal total cholesterol content of the membrane could also be associated with a local (lateral) depletion or reduction of the sterol in one or both of the two monolayers, thereby inducing changes in kinetic parameters of Na-Li exchange.

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