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POTASSIUM TRANSPORT AND LIPID COMPOSITION IN MAMMALIAN RED BLOOD CELL MEMBRANES

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Summary

Potassium influxes in red cells from eight species have been found to follow an exponential relationship with membrane phosphatidylcholine and sphingomyelin content. This relationship with membrane phospholipid patterns was found to exist with both ouabain sensitive and insensitive fractions of potassium transport. When published values of chloride and phosphate permeabilities were compared with potassium permeabilities, correlations were found in seven out of nine of the species studied. On the basis of these findings it appears that potassium, phosphate, and chloride permeabilities in red blood cells of most species are related to the membrane phosphatidylcholine and sphingomyelin content; that is, membrane permeabilities increase with increasing amounts of phosphatidylcholine and decrease with increasing amounts of sphingomyelin. These results indicate that the membrane lipid is an important factor in transport processes in mammalian red blood cells.

Introduction

Differences in membrane permeability in various red cells have been reported for nonelectrolytes such as glycerol, erythritol, and urea and for anions such as phosphate, chloride, and sulfate [1—6]. The differences in permeabilities for phosphate have been related to the membrane phospholipid patterns [5]. Large variations in glycerol permeability in erythrocytes from eight mammalian species are reported not to correlate with differences in phospholipid composition; this is probably due to the existence of a facilitated diffusion glycerol transport system in red blood cells of several mammalian species [7]. In contrast to these passive transport systems, potassium transport represents an

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energy dependent process. Potassium transport is known to be protein mediated. In this paper the role of the membrane lipid composition in potassium transport is examined.

Potassium transport depends on both the external and internal cation concentrations [8]. It has been found that the concentrations of potassium and sodium are relatively constant in plasmas of various species (see Table I). As is shown in Table I, the internal red cell potassium concentrations were high (106—86 mM/l of cells) and sodium concentrations were low (18—6 mM/l of cells) in all of the species studied except for bovine. The potassium influx is extremely low in bovine red cells in comparison to pig, human, rabbit, guinea pig, and rat cells. Bovine cells are similar to sheep and goat cells with respect to their low potassium influxes (see Table I) and membrane lipid composition [9]. It has been found in sheep red cells that high intracellular levels of sodium and low levels of potassium yield near maximum potassium influx values [8]. As

TABLE I

CATION COMPOSITION AND TRANSPORT PROPERTIES OF RED CELLS AND PLASMA

Species	Plasma		Cellular [c]		K^+-Influx [c]		Ref.
	$[K]_0$ (mmol/l)	$[Na]_0$	$[K]_i$ (mmol/l cells)	$[Na]_i$	Total (mM/l Cells per h)	With Ouabain	
Sheep			98	13	0.58 ± 0.05	0.08 ± 0.02	a
			85	15	0.57	0.04	8
	5	151					22
Bovine			17	70	0.41 ± 0.08	0.14 ± 0.04	a
			27	65	0.29	0.12	23
	6	145					24
Goat			86	18	1.0 ± 0.14	0.15 ± 0.02	a
			—	—	—	—	—
	4	190					25
Horse			106	9	0.83 ± 0.05	0.09 ± 0.03	a
			88	—	1.3	—	26
	6	154					22
Pig			101	8	1.4 ± 0.12	0.21 ± 0.05	a
			100	11	0.78	0.04	27
	6	149					28
Human			92	10	1.8 ± 0.03	0.26 ± 0.03	a
			89	11	1.6	0.44	29
	4	140					30
Rabbit			86	10	3.0 ± 0.13	0.19 ± 0.08	a
			96	10	2.10	0.31	29
	6	150					22
Guinea Pig			98	8	5.8 ± 0.61	0.71 ± 0.07	a
			105	15	3.32	0.48	31
	6	—					32
Rat			104	6	4.8 ± 0.70	3.4 ± 0.90	a
			106	4	5.0 [b]	3.5 [b]	33
	6	152					22

[a] Average values obtained in a medium described by Sachs and Welt, [10].

[b] Rubidium influx

[c] $[K]_i$, $[Na]_i$, and K^+-influx were measured on the same cells.

a result of (1) the relative low potassium influxes and (2) transport system operating near maximum influx, the internal cation composition of bovine red cells should not alter the overall relationship between transport and lipid patterns reported in this communication. High potassium sheep cells were chosen to study since all other cells which were studied had high internal potassium and low sodium concentrations except for bovine cells. Since previously reported values for potassium influx have been obtained in different laboratories under varying external conditions, it was decided to compare mammalian red cells under identical external conditions and under physiological internal conditions.

Methods

All experiments were performed with blood freshly drawn with a syringe treated with heparin (Upjohn Co., Kalamazoo, Mich.). Plasma and white cells were removed by aspiration after the blood had been centrifuged. The cells (2 ml) were then washed three times at 37°C in 10 ml of a medium described by Sacks and Welt [10] (Solution SW). Solution SW contained the following: 29 mM glycylglycine, 6 mM $MgCO_3$, 130 mM NaCl, 5 mM KCl, 0.45 mM Na_2HPO_4, 0.85 mM $NaH_2PO_4H_2O$, 11 mM dextrose (pH 7.4). The medium used in this study was isosmotic since cell volumes in this medium were the same as cell volumes measured in plasma.

The procedures for measurement of potassium influx were similar to those described by Gunn and Tosteson [11]. Aliquots (0.2 ml) of packed cells were incubated for 45 min in 10 ml Erlenmeyer flasks containing 2 ml of Solution SW at 37°C. Ouabain from a 10^{-2} stock solution in isotonic NaCl was added to half of the incubation flasks to give a final concentration of 10^{-4} M. Samples were taken at 30 and 60 min after the $^{42}K^+$ isotope had been added (10 μCi of $^{42}K^+$) and the uptake was found to be linear. The samples were transferred to centrifuge tubes containing 2.0 ml of dibutyl phthalate with 8 ml of isotonic $MgCl_2$ layered over it. The tubes were immediately centrifuged at 12 000 × g for 1 min. The pellet and dibutyl phthalate layer were not disturbed when the $MgCl_2$ solution was removed by aspiration and fresh $MgCl_2$ was layered onto the phtalate. After several minutes both the $MgCl_2$ and phthalate were completely removed and the cell pellet was lysed with 2.9 ml of hemolyzing fluid (0.61 mM $K_3Fe(CN)_6$, 0.77 mM KCN, 1 mM KH_2PO_4, 0.5 ml non-ion-ox (A.S. Aloe Co., St. Louis, Mo.) 0.004 M CsCl per liter of solution, pH 7.4). Radioactivity was measured in a well-type crystal scintillation spectrometer, and the hemoglobin concentration of the samples relative to that of the original packed cells was determined by the cyanmethemoglobin method. The potassium influx was calculated as previously described by Gunn and Tosteson [11].

Cellular volumes in plasma and Solution SW were calculated from hematocrit measurements and cell number was determined by counting cells in a hemocytometer. Since extracellular trapped volumes are small, they were neglected.

Results

The total potassium influx values in red cells of eight of the nine species studied in our laboratory were found to correlate with membrane phospatidyl-

choline and sphingomyelin composition (see Fig. 1). The sum of these two phospholipid compositions has been reported to be constant in membranes of mammalian red cells [9], and therefore, if a correlation is observed between transport and phospatidylcholine content, an inverse relationship will necessarily exist with sphingomyelin levels as shown in Fig. 1. Potassium transport in the various species was also compared with the membrane phosphatidylcholine pattern on the basis of surface area. The fluxes were computed using surface areas measured by Deuticke and Gruber [5]. The relationship between potassium transport and phosphatidylcholine composition still appeared. The major reason this relationship was not significantly changed by using a surface area basis for comparison was that the permeabilities varied by almost a factor of 40. This was relatively large when compared to the differences in volume-surface area ratios which varied by less than a factor of 2. These ratios were used to express the potassium transport on the basis of surface area.

The relationship between potassium permeabilities and membrane phosphatidylcholine composition was not linear but appeared to be exponential. For this reason the potassium transport data and phosphatidylcholine pattern were

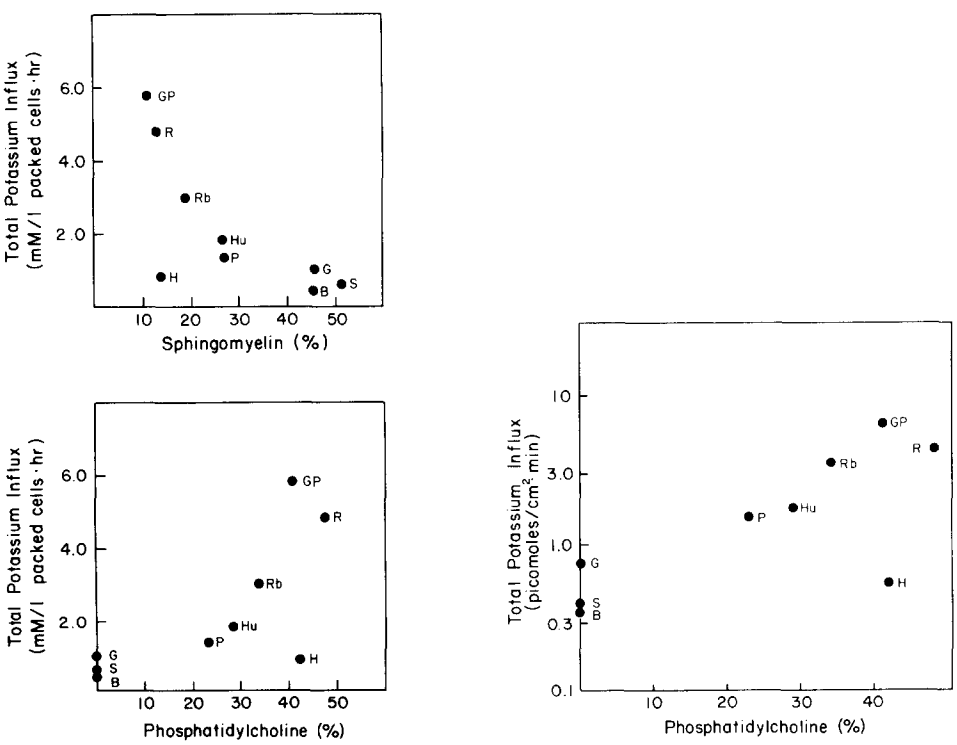

Fig. 1. Relationship between total potassium influx and sphingomyelin and phosphatidylcholine content in mammalian red blood cells. Phospholipid values were taken from Nelson [9] and they are expressed as percentages of the sums of the membrane phospholipids. (R, rat: GP, Guinea pig; H, Horse; Rb, Rabbit; Hu, Human; P, pig; B, bovine; S, sheep).

Fig. 2. Total potassium influx in red cells compared with membrane phosphatidylcholine levels using a semilogarithmic plot. The fluxes were computed using surface areas reported by Gruber and Deuticke [5]. Abbreviations as in legend to Fig. 1.

compared using a semilogarithmic plot as shown in Fig. 2. In this graph it appears that eight species follow the phosphatidylcholine composition exponentially. The only exception is the horse red cell for which other factors seem to control potassium permeability. The cardiac glycoside, ouabain, is known to reduce sodium and potassium transport in mammalian red blood cells [15]. In the eight species following the lipid pattern both the ouabain sensitive and insensitive fractions contributed to the relationship between transport and lipid composition (see Fig. 2).

In this study potassium transport in red cells was also compared with relative amounts of membrane glycolipid, phospholipid, cholesterol, and the molar ratio of cholesterol and phospholipid using the lipid composition data of Rouser et al. [16]. No correlations could be found. Potassium transport was compared with relative amounts of phospholipids in the nine species studied using the published data of Nelson [9] and Rouser et al. [16]. Again, no correlations were found with lysophosphatidylcholine, phosphatidylinositol, phosphatidylserine, phosphatidylethanolamine, or phosphatidic acid content.

Potassium influxes measured in other laboratories under varying conditions are compared in Table I with the values obtained in our laboratory using near-physiological concentrations of external potassium and sodium. Although several of the influx values for the nine types of red cells differ from those previously reported, the relationships observed between values of potassium influx and membrane phosphatidylcholine or sphingomyelin composition exist for both sets of values.

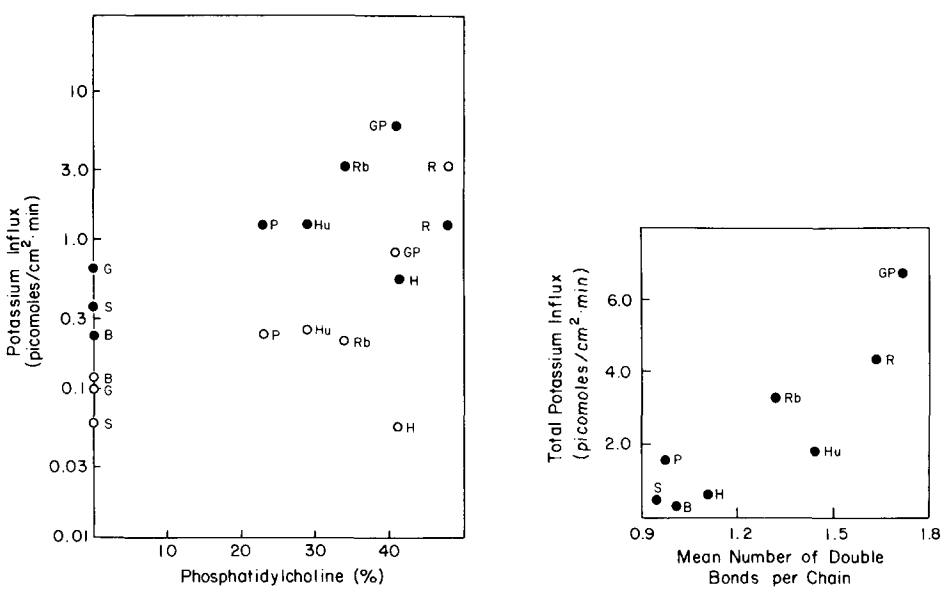

Fig. 3. Comparisons of ouabain sensitive potassium influx (closed circles) and ouabain insensitive potassium influx (open circles) in red cells with membrane phosphatidylcholine composition using a semilogarithmic plot. Abbreviations as in Fig. 1.

Fig. 4. Comparisons of total potassium influx in red cells with mean number of double bonds. The mean number of double bonds are calculations of Wessels and Veerkamp [7]. Abbreviations as in Fig. 1.

Discussion

In eight species potassium transport was found to follow sphingomyelin and phosphatidylcholine patterns (see Fig. 1). It has been reported that red cell membrane phospholipids distribute asymmetrically with sphingomyelin and phosphatidylcholine on the outside half of the lipid bilayer and, with the phosphatidylethanolamine and phosphatidylserine primarily on the inside [17]. The red cell membrane content of phosphatidylserine and phosphatidylethanolamine is relatively constant when compared with variations in sphingomyelin and phosphatidylcholine composition. Sphingomyelin contains a high percentage of saturated long-chain fatty acids [19]. Evidence that chain length and the extent of saturation of the phospholipid fatty acids influence membrane permeability has been provided by studies on model membranes [18]. Double bonds produce kinks in the fatty acid chains which reduce the London-van der Waals force of attraction between neighboring molecules. On the other hand, longer fatty acid chains have more molecular regions which can interact, resulting in stronger forces of attraction. Therefore, a membrane whose lipids are highly unsaturated and have medium-length fatty acid chains will not be as densely packed as one with long chain fatty acids with a high degree of saturation [19]. Thus, one would expect that large amounts of sphingomyelin would restrict transport proteins resulting in reduced permeabilities. For these reasons, the potassium permeabilities of the red cells were compared with the mean number of double bonds, the mean number of carbon atoms, and the mean "effective" length of the hydrocarbon chains. Relationships were found with the mean number of double bonds and the "effective" chain length calculated by Wessels and Veerkamp [7]. However, no relationship was observed between potassium transport and the mean number of carbon atoms of the hydrocarbon chains. The problems of measuring and calculating and "effective" length for the membrane hydrocarbon chains has been previously discussed [20,21]. In brief, capacitance measurements, X-ray studies, and theoretical calculations have resulted in drastically different values for an "effective" length for the hydrocarbon chains. Clearly, the mean number of carbon atoms of the hydrocarbon chains is not an adequate parameter for the evaluation of the role of hydrocarbon chain length in transport processes [20,21].

Since Deuticke and Gruber [5] have also found a relationship between phospholipid patterns and phosphate transport, a comparison of potassium and phosphate transport in red cells has been made. It was found that as phosphate influx increased potassium influx also increased. Another comparison has been made between potassium and chloride transport systems using the chloride fluxes reported by Wieth et al. [4] There is a relationship in seven of the species, but the results from bovine cells fall very much off the line.

There is ample evidence that the membrane protein, $(Na^+ + K^+)$-ATPase, is able to utilize the chemical energy of ATP to transport sodium and potassium. The $(Na^+ + K^+)$-ATPase is part of the 95 000 dalton band (band III) of the protein seen on sodium dodecyl sulphate gel electrophoresis. The interaction of $(Na^+ + K^+)$-ATPase with its lipid environment has been studied in reconstituted model membranes [34]. This membrane protein as well as rhodopsin requires lipid for its functional activity [35,36]. From results reported in this paper, it

is possible that the lipid environment of the membrane transport proteins affects transmembrane fluxes in red blood cells, since membrane permeabilities increased with the increase in content of phosphatidylcholine and decreased with the increase in sphingomyelin content. However, the effect of lipid composition on potassium transport is possibly due to direct changes in membrane passive transport. Potassium influxes were measured under steady-state conditions and under these conditions "active" K^+ influx is a function of the "leak" flux. This interpretation is the more probable since the known effects of lipids on the (Na^+ + K^+)-pump do not support a direct effect by lecithin and sphingomyelin [37]. In addition, it is known that ruminants and man have drastically different numbers of active transport sites measured as specific ouabain binding sites. The ruminants also have a higher affinity of the pump for potassium [38].

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