Thallium and Rubidium Permeability of Human and Rat Erythrocyte Membrane

I. A. SKULSKII, V. MANNINEN* and V. V. GLASUNOV

Institute of Evolutionary Physiology and Biochemistry, Academy of Sciences of the USSR, pr. M. Thorez 44, 194 223 Leningrad, USSR

Abstract. Transport of Tl^+ and Rb^+ in human and rat erythrocytes was investigated in the presence of ouabain. The chloride-dependent cotransport of Tl^+, Rb^+ and Na^+ was precluded by replacement of Cl^- by NO_3^-. The inward and outward rate constants for the residual fluxes of the cations were determined by measuring the transport of ^{204}Tl and ^{86}Rb in double label experiments. The rate of passive transport of Tl^+ exceeded that of Rb^+ by one-two orders of magnitude in human as well as rat erythrocytes. The membrane barrier which contributes to the maintenance of ion gradients was shown not to be a barrier for Tl^+ which easily penetrates the membrane by an unknown mechanism. In rat erythrocytes the barrier for Rb^+ was 10—15 times weaker than that in human red blood cells, while the corresponding ratio of rat/human Tl^+ permeabilities was about 1.8—2.0. It follows that Tl^+ permeability is only slightly affected by factors modifying the permeability to alkali cations. The increase of temperature from 20° to 37 °C resulted in a three-fourfold stimulation of the passive transport of Tl^+ both in human and rat erythrocytes. The movement of Tl^+ and Rb^+ through the erythrocyte membrane differed substantially from their diffusion along the excitable membrane channels characterized both by poor Tl^+/K^+ selectivity and weak temperature dependence.

Key words: Thallium — Rubidium — Erythrocytes — Membrane permeability

Introduction

The attention paid by physiologists to Tl^+ can be explained both by the high toxicity and the ability of the element to mimic K^+. The similarity between Tl^+ and K^+ is based on the closeness of the ionic radii (0.147 and 0.133 nm, respectively) (Nightingale 1959). A certain specificity in the behavior of Tl^+ as compared to alkali metal cations, can to some extent be ascribed to a difference

* Present address: The Wihuri Research Institute, SF-00140, Helsinki, Finland

in molecular polarizability. Thus, Tl^+ and Rb^+ have identical radii, but the polarizability of Tl^+ (5.2×10^{-24} cm^3) is considerably higher than that of Rb^+ (1.8×10^{-24} cm^3) (Lee 1977).

The similarity between Tl^+ and K^+ (Rb^+) depends on the system under study. In potassium channels of excitable membranes the discrimination of Tl^+, Rb^+ and K^+ is rather poor (Mullins and Moore 1960; Landowne 1975; Edwards and Vyskočil 1984). On the contrary, in the erythrocyte membrane the passive Tl^+ permeability, being of the same order as that in nerve and muscle cell membranes, is one-two orders of magnitude higher than the permeability to K^+ (Skulskii et al. 1973; 1978). Such a high Tl^+/K^+ selectivity of erythrocyte membrane can be explained in two different ways: (i) Tl^+ and K^+ permeate the erythrocyte membrane via a common structure characterized by a high ratio of Tl^+/K^+ permeabilities, or (ii) Tl^+ and K^+ cross the membrane by different routes. A direct study of Tl^+ and K^+ (Rb^+) competition seems to be a difficult task since an elevated Tl^+ concentrations may damage the membrane.

In the present work the kinetics of trace amounts of ^{204}Tl and ^{86}Rb were studied in human as well as rat erythrocytes. The latter are known to be about 10 times more permeable to K^+ as compared to human red blood cells (Kirk 1977). If the first explanation is true, Tl^+ and Rb^+ (K^+) permeabilities will vary in parallel, and Tl^+ and Rb^+ can be assumed to leak through a common membrane structure. Alternatively, if a considerable change in K^+ permeability is not followed by an adequate alteration of Tl^+ passive fluxes, independent mechanisms of Tl^+ and K^+ transmembrane movements have to be suggested.

Materials and Methods

Fresh heparinized blood was centrifuged at $3000 \times g$ for 10 min to separate erythrocytes from plasma and buffy coat. The cells were washed 2—3 times with 10 volumes of a buffered saline medium and subsequently resuspended in the same solution. The composition of the medium was as follows (mmols/l): $NaNO_3$ 135, KNO_3 5, $TrisNO_3$ 10, pH 7.4. The incubation medium contained no Cl^- to preclude both TlCl precipitation and Cl^--dependent contransport of Tl^+, Rb^+ and Na^+ (Geck and Heinz 1986; Rangachari and McWade 1986). The final haematocrit was 5%. After preincubation of the cells with 1 mmol/l ouabain the uptake experiment was started by adding to the incubation medium 10 μCi/ml ^{86}Rb and 5 μCi/ml ^{204}Tl. Samples of the cell suspension were taken at specified intervals and immediately cooled by mixing with 10-fold excess of ice-cold isotonic $Mg(NO_3)_2$ solution. These samples were centrifuged and washed once in the counting tubes with the same saline preparation. Reverse movement of tracers during centrifugation and washing was negligible.

In the efflux experiments, the unwashed erythrocytes were first incubated at 40—50% haematocrit overnight at 4 °C with 100μCi/ml ^{86}Rb and 50 μCi/ml ^{204}Tl in order to label the intracellular medium with both tracers simultaneously. The labelled cells were washed with ice-cold base medium and the efflux experiment was initiated by mixing the cells at 20 °C or 37 °C with the same medium at 0.5—1.0% haematocrit. Aliquots were taken at specified intervals into 10-fold volumes of

Table 1. Kinetics of $^{86}Rb^+$ and $^{204}Tl^+$ uptake in human and rat erythrocytes at 37 °C in the presence of $5 \cdot 10^{-4}$ mol/l ouabain

Species	k_{in} (Rb^+) (min^{-1})	k_{in} (Tl^+) (min^{-1})	$\frac{k_{in}(Tl^+)}{k_{in}(Rb^+)}$	$\frac{k_{in}\text{ (rat)}}{k_{in}\text{ (human)}}$	
				Rb^+	Tl^+
Human	0.000 48	0.071	148	15.4	2.3
Rat	0.007 4	0.163	22		

Table 2. Kinetics of $^{86}Rb^+$ and $^{204}Tl^+$ efflux from human and rat erythrocytes at 37 °C

Species	$k_{out}(Rb^+)$ (min^{-1})	$k_{out}(Tl^+)$ (min^{-1})	$\frac{k_{out}(Tl^+)}{k_{out}(Rb^+)}$	$\frac{k_{out}\text{ (rat)}}{k_{out}\text{ (human)}}$		$\frac{k_{in}}{k_{out}} = r_{ss}$	
				Rb^+	Tl^+	Rb^+	Tl^+
Human	0.000 23	0.045	196	10	1.8	2.09	1.58
Rat	0.002 3	0.081	35			3.21	2.01

ice-cold 110 mmol/l $Mg(NO_3)_2$, centrifuged and washed once with the same solution. Both in the uptake and in efflux experiments 10—15 aliquots were taken during 1 to 2 h of observation. Radioactivity of the samples was determined with an automatic, dual channel well type scintillation spectrometer (Wallak, Decem). The ^{86}Rb and ^{204}Tl tracers were supplied by Radiochemical Centre, Amersham, U.K. The specific activity of the tracers was sufficiently to provide low concentrations of cold Rb^+ and Tl^+ (about 10^{-5}—10^{-6} mol/l).

The inward rate constants were calculated from initial slopes of the uptake curves (Skulskii et al. 1978). The accumulation of tracers was linearly related to time, i.e. $k_{in} = (r_2 - r_1)/(t_2 - t_1)$, where r_1 and r_2 represent the cell / medium distribution of the tracers at times t_1 and t_2. The outward rate constants were determined from a semilogarithmic plot: $k_{out} = \ln(A_2/A_1)/(t_2 - t_1)$, where A_1 and A_2 represent the cell radioactivity at times t_1 and t_2. Under steady state condition the tracer rate constants were related to the cell/medium distribution, r_{ss}, by the equation $k_{in}/k_{out} = r_{ss}$. In principle, the rate constants are concentration dependent, but in our experiments the passive fluxes of the cations were far from being saturated.

Results and Discussion

Tables 1—3 show the results obtained in out of 3—5 parallel double label experiments ($^{86}Rb + {}^{204}Tl$). The standart deviation did not exceed 10 % of the mean. Both in human and rat erythrocytes the inward rate constants of Tl^+ greatly exceeded those of Rb^+ (Table 1). The Tl^+/Rb^+ ratios of inward rate

Table 3. Effect of temperature on the kinetics of $^{204}Tl^+$ passive transport in human and rat erythrocytes

Species	t °C	k_{in} (min^{-1})	$\frac{k_{in}\,(37\,°C)}{k_{in}\,(20\,°C)}$	k_{out} (min^{-1})	$\frac{k_{out}\,(37\,°C)}{k_{out}\,(20\,°C)}$
Human	20	0.022	3.23	0.0124	3.39
	37	0.071		0.0420	
Rat	20	0.052	3.13	0.0178	3.89
	37	0.163		0.0693	

constants were 148 and 22 for human and rat erythrocytes, respectively. A lower ratio in the rat erythrocytes is the result of the disproportionate alteration of Rb^+ and Tl^+ permeabilities.

The rat/human ratio of Rb^+ rate constants was about 15, while an only 2-fold increase of Tl^+ permeability could be observed. The efflux rate constants of Tl^+ exceeded those of Rb^+ by 1—2 orders of magnitude (Table 2). The Tl^+/Rb^+ permeability ratios were 196 and 35 for human and rat erythrocytes, respectively. In accordance with the results of the uptake experiments, the 10-fold increase of the Rb^+ permeability in rat erythrocytes was not accompanied by an equivalent rise of the Tl^+ efflux rate.

The ouabain-insensitive K^+ influx in erythrocytes of eight mammalian species has been reported to increase with increasing amount of phosphatidylcholine and decrease with the increasing amounts of sphingomyelin (Kirk 1977). Tl^+ leak may also depend on the membrane composition and phase transition (Miller 1985); nevertheless the Tl^+ pathways seems to be inaccessible to K^+ (Rb^+).

The temperature increase from 20° to 37 °C greatly facilitated the Tl^+ passive transport both in human and in rat erythrocytes (Table 3). Comparable values of activation energy were measured in studies of the diffusion of univalent cations across the lamellae of swollen phospholipids (Bangham et al. 1965). A high activation energy indicates that the passive leak of Tl^+ and K^+ across the erythrocyte membrane is not merely diffusion through K^+ channels known to operate in excitable membranes. In contrast to erythrocyte membranes the former are characterized both by a poor Tl^+/K^+ selectivity and by a weak temperature dependence (Landowne 1975; Edwards and Vyskočil 1984).

According to the fixed charge model (Passow 1969), the erythrocyte membrane barrier contains positively charged groups which repel cations. However, because of the closeness of their crystal and hydrated radii (Nightingale 1959; Mullins and Moore 1960) Tl^+ and K^+ cannot be selected by pure electrostatic

repulsion forces. The high permeability of erythrocyte membranes to Tl^+ has been ascribed to a hypothetical electrically silent flux of ionic pairs, e.g. $(TlNO_3)°$, (TlOH)° etc. (Gutknecht 1983; Izatt et al. 1986), but the association constants of the neutral species formation seem to be too low (Lee 1972). Moreover, both the rate of Tl^+ passive transport and the cell/medium stationary distribution of Tl^+ in a suspension of human erythrocytes were found to depend on the membrane potential in accordance with the expected behavior of a permeant cation (Skulskii and Manninen 1981). The membrane barrier preventing loss of cell K^+ proved not to be a barrier to Tl^+ which crosses the erythrocyte membrane via a specific pathway. The Tl^+ and K^+ permeabilities may vary independently despite some factors (pH, t°) which similarly affect the penetration of both cations (Skulskii et al. 1973; 1978; Skulskii and Manninen 1981).

References

Bangham A. D., Standish M. M., Watkins J. C. (1965): Diffusion of univalent ions across the lamellae of swollen phospholipids. J. Mol. Biol. **13,** 232—252

Edwards C., Vyskočil F. (1984): The effects of the replacement of K^+ by Tl^+, Rb^+ and NH_4^+ on the muscle membrane potential. Gen. Physiol. Biophys. **3,** 259—264

Geck P., Heinz E. (1986): The Na-K-2Cl cotransport system. J. Membrane Biol. **91,** 97—105

Gutknecht J. (1983): Cadmium and thallium ion permeation through lipid bilayer membranes. Biochim. Biophys. Acta **735,** 185—188

Izatt R. M., Bruening R. L., Clark G. A., Lamb J. D., Christensen J. J. (1986): Effect of co-anion on DC18C6-mediated Tl^+ transport through an emulsion liquid membrane. J. Membrane Sci. **28,** 77—86

Kirk R. G. (1977): Potassium transport and lipid composition in mammalian red blood cell membranes. Biochim. Biophys. Acta **464,** 157—164

Landowne D. (1975): A comparison of radioactive thallium and potassium fluxes in the giant axon of the squid. J. Physiol. (London) **252,** 79—96

Lee A. G. (1972): The coordination chemistry of thallium (I). Coordination Chem. Revs. **8,** 289—349

Miller I. R. (1985): Permeability of vesicular phospholipid bilayer membranes to thallium and its facilitation. Biochim. Biophys. Acta **817,** 199—207

Mullins L. J., Moore R. D. (1960): The movement of thallium ions in muscle. J. Gen. Physiol. **43,** 759—773

Nightingale E. R. (1959): Phenomenological theory of ion solvation. Effective radii of hydrated ions. J. Phys. Chem. **63,** 1381—1387

Passow H. (1969): Passive ion permeability of the erythrocyte membrane. Progr. Biophys. Mol. Biol. **19,** 422—467

Rangachari P. K., McWade D. (1986): Ouabain-insensitive, halide-sensitive Tl^+ uptake by canine illiac arteries. Biochim. Biophys. Acta **854,** 251—256

Skulskii I. A., Manninen V. (1981): Effects of membrane potential on passive transport of Tl^+ in human red blood cells. Acta Physiol. Scand. **111,** 342—348

Skulskii I. A., Manninen V., Järnefelt J. (1973): Interaction of thallous ions with the cation transport in erythrocytes. Biochim. Biophys. Acta **298,** 702—709

Skulskii I. A., Manninen V., Järnefelt J. (1978): Factors affecting the relative magnitudes of the ouabain-sensitive and ouabain-insensitive fluxes of thallium ion in erythrocytes. Biochim. Biophys. Acta **506,** 233—241

Final version accepted July 7, 1989