Species-dependent Differences in the Influence of Ionic Strength on Potassium Transport of Erythrocytes. The Role of Membrane Fluidity and Ca$^{2+}$

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Abstract. The passive Rb$^+$ (K$^+$) efflux from erythrocytes of seven mammalian species was investigated in solutions of physiological and low ionic strength. Furthermore the fluidity of the erythrocyte membrane in the same solutions was estimated by measuring the ESR order parameter. The rate constant of Rb$^+$ (K$^+$) efflux in solution of high ionic strength could be correlated with the order parameter obtained and with the mean number of double bonds to the membrane phospholipid fatty acids. The same relationships could be observed for the low ionic strength solutions if the values for human erythrocytes were excluded. The appearance of Na$^+$, K$^+$, Cl$^-$ cotransport to a significant extent, only in human erythrocytes, was supposed to be the reason for this different behaviour of human red blood cells. It was demonstrated that the strong increase of the Rb$^+$ (K$^+$) efflux rate constant for human erythrocytes in low ionic strength solution is not due to Ca$^{2+}$, as quinine treatment and replacement of all external potassium, both inhibiting the Ca$^{2+}$-induced K$^+$ efflux, did not abolish the increase of (Rb$^+$) K$^+$ efflux in solutions of low ionic strength.

Key words: Rb$^+$ (K$^+$) efflux — Erythrocytes — Mammalian species — Membrane fluidity — Ca$^{2+}$-induced K$^+$ efflux

Introduction

Differences in ionic transport between the red blood cells of various species have been reported for nonelectrolytes such as glycerol and erythritol, for anions such as phosphate and chloride, and for the active transport of potassium (de Gier...
The majority of these authors tried to relate these differences to phospholipid patterns. The importance of the lipid composition for ion transport, particularly the change of permeability around the phase transition temperature, is well established (Block et al. 1975). For various membrane associated enzymes a specific lipid composition is necessary to maintain their conformation and function (Roelofsen 1977; Abeywardena and Charnock 1983). In liposomes the structure of the phospholipid acyl chains determines the permeation of nonelectrolytes (de Gier et al. 1968). McLean et al. (1988) emphasized the importance of membrane fluidity for the post-transfusion survival of erythrocytes.

Erythrocytes from various mammalian species have been investigated, and so the lipid composition of the phospholipids as well as the structure of the acyl chains were varied without any artificial modification of the cells. These variations can be expected to result in a change of what is called membrane fluidity. This term is used although there are different interpretations of the physical meaning depending on the method of the fluidity determination.

In 1939, Davson reported that erythrocytes suspended in isotonic solutions of low ionic strength lose cations. Later these findings were precised and interpreted as representing a change of $K^+$ permeability of human erythrocytes dependent on the transmembrane potential (Donlon and Rothstein 1969). It could be shown that a change in permeability is not necessarily required to explain this behavior if the inner and the outer surface potentials are introduced in the Goldman flux equation (Bernhardt et al. 1984).

The effect of a reduction of the extracellular ionic strength on $Rb^+$ ($K^+$) efflux from erythrocytes of various mammalian species was investigated. The relationship of the species-dependent differences of the rate constants observed in solutions of physiological and low ionic strength to membrane fluidity and $Ca^{2+}$-induced $K^+$ efflux are discussed.

Materials and Methods

1. Erythrocyte collection and preparation: With the exception of human erythrocytes, where 2-day-old whole blood (citrate preserved) of the 0 Rh$^+$ group was used, the blood of the chosen animals was drawn by venipuncture with heparin (1.5 mg per 10 ml blood) as anticoagulant. Serum and buffy coat were removed by centrifugation ($2000 \times g, 8 \text{ min, } 293 \text{ K}$) followed by aspiration of the upper layers. Subsequently the erythrocytes were washed twice ($2000 \times g, 8 \text{ min, } 293 \text{ K}$) in the standard solution of high ionic strength containing (mmol/l): NaCl 141.3, KCl 5.7, glucose 5.0, and $Na_2HPO_4/NaH_2PO_4$ 5.8, pH = 7.4.

2. Determination of the rate constant of $86-Rb^+$ efflux: The method of continuous efflux measurements was described in detail elsewhere (Bernhardt et al. 1984). Briefly, 0.08 ml of Rb-86-Cl ($Rb^+$...
Table 1. Rate constants $k$ of Rb$^+$-efflux of various mammalian species in solution of high and low (NaCl + KCl)-concentration

<table>
<thead>
<tr>
<th>Species</th>
<th>$\text{(NaCl + KCl)} = 147 \text{ mmol/l}$</th>
<th>$\text{(NaCl + KCl)} = 5.7 \text{ mmol/l}$</th>
<th>$k$ $5.7/k$ 147</th>
<th>Ca$^{2+}$ induced K$^+$ efflux</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cow</td>
<td>$7.0 +/− 0.6 (5)$</td>
<td>$8.7 +/− 1.4 (9)$</td>
<td>$1.24 \text{ n.s.}$</td>
<td>$− (a, b)$</td>
</tr>
<tr>
<td>Pig</td>
<td>$3.3 +/− 1.0 (6)$</td>
<td>$5.0 +/− 1.9 (6)$</td>
<td>$1.52 \text{ n.s.}$</td>
<td>$+ (b, c)$</td>
</tr>
<tr>
<td>Rabbit</td>
<td>$8.4 +/− 2.8 (8)$</td>
<td>$12.3 +/− 1.2 (9)$</td>
<td>$1.46 \text{ s.}$</td>
<td>$+ (b, c, d)$</td>
</tr>
<tr>
<td>Horse</td>
<td>$3.8 +/− 1.0 (5)$</td>
<td>$9.1 +/− 3.9 (5)$</td>
<td>$2.40 \text{ s.}$</td>
<td>$− (c)$</td>
</tr>
<tr>
<td>Rat</td>
<td>$13.6 +/− 2.6 (5)$</td>
<td>$39.8 +/− 9.7 (6)$</td>
<td>$2.93 \text{ s.}$</td>
<td>$+ (a, b, c, e)$</td>
</tr>
<tr>
<td>Cat</td>
<td>$6.9 +/− 1.1 (6)$</td>
<td>$23.1 +/− 3.2 (5)$</td>
<td>$3.33 \text{ s.}$</td>
<td>$+ (d)$</td>
</tr>
<tr>
<td>Man</td>
<td>$8.1 +/− 0.4 (7)$</td>
<td>$63.6 +/− 4.2 (8)$</td>
<td>$7.85 \text{ s.}$</td>
<td>$+ (a, b, c, d, e)$</td>
</tr>
</tbody>
</table>

$T = 310 K$; pH = 7.4; osmolarity = 290 mOsmol/l; means $+/− 1 \text{ S. D.}$

$a$: Jenkins and Lew (1973); $b$: Allan and Michell (1977); $c$: Eaton et al. (1978); $d$: Miner et al. (1983); $e$: Dunker and Passow (1953). The figures in brackets indicate number of experiments. “s.” and “n. s.”, respectively, denote significant or non-significant differences at $p = 0.05$ (Welch’s $t$-test).

as K$^+$ indicator) were added to 1 ml erythrocyte suspension (hematocrit = 35%), giving a maximum value of activity of 13.7 MBq. The mixture was incubated for 2 hours at 310 K. The loss of radioactivity was detected continuously by means of scintillation counting. The cell suspension was poured into a diffusion chamber which was closed by a milipore filter to allow ions and molecules to diffuse but to restrain the cells. The flushing solution was either the standard solution or a solution of low ionic strength containing (mmol/l): KCl 5.7, glucose 5.0, sucrose 262.2, Na$_2$HPO$_4$, NaH$_2$PO$_4$ 5.8, pH = 7.4. The experiments were always performed at 310 K. The rate constant was calculated as the negative slope of the linear regression line obtained by semilogarithmic plot of counts against time, and is expressed in terms of [l/min].

3. ESR measurements: The fatty acid spin label used for all experiments was 2-(3-carboxypropyl)-2-decyl-4,4-dimethyl-3-oxazolidinyloxyl (1(10.3)). A thin film of the label was prepared on the wall of a glass vessel by dissolving 0.08 mg of the label in carbon tetrachloride and evaporating the solvent with a stream of nitrogen. 2 ml erythrocytes in standard solution (hematocrit = 45%) were added and then carefully shaken at room temperature for 20 minutes. This procedure ensures that interactions between the spin label molecules are avoided (Herrmann et al. 1982). Subsequently the erythrocytes were separated from the solution by centrifugation (1000 x g, 5 min) and washed twice with 2 ml of either the standard solution or the solution of low ionic strength (1000 x g, 8 min). ESR spectra were recorded on an ESR spectrometer (ZWG, Academy of Science, GDR), with a flat quartz cell for aqueous solutions. All measurements were carried out with the following settings: Microwave power = 20 mW, modulation amplitude = 0.4 mT, scan time = 6.6 min, time constant = 0.03 s, temperature = 310 K. The temperature was measured with an accuracy of $+/− 0.1 K$ with a small thermistor inserted in the sample cell. The order parameter was calculated according to Griffith and Jost (1976) using the following $T$-tensors: $T_{xx} = 0.695 \text{ mT}$, $T_{yy} = 0.535 \text{ mT}$, $T_{zz} = 3.300 \text{ mT}$. 
Fig. 1. The order parameter of the spin label 1(10,3) incorporated in the erythrocyte membrane of various mammalian species in high and low ionic strength solutions. Open columns: (NaCl + KCl) = 147 mmol/l; hatched columns: (NaCl + KCl) = 5.7 mmol/l; T = 310 K, pH = 7.4, osmolarity = 290 mOsmol/l.

Results

The Rb$^+$ (K$^+$) efflux from erythrocytes of the following seven mammalian species was investigated: man, cat, rat, horse, pig, rabbit, and cow. The rate constants for Rb$^+$ (K$^+$) efflux in the standard solution of physiological ionic strength (NaCl + KCl = 147 mmoles/l) and in the solution of low ionic strength (NaCl + KCl = 5.7 mmoles/l) are shown in Table 1, also giving the ratio of the rate constant in low ionic strength solution to that in the standard solution (k5.7/k147). The appearance of the Ca$^{2+}$-induced K$^+$ efflux (Gardos 1958) is also indicated. It is noteworthy that there was no correspondence. The main differences in membrane lipids between the erythrocytes of various mammalian species concern the relative phospholipid contents (Nelson 1967) as well as the structure of the acyl chains of their fatty acids (Wessels and Veerkamp 1973). An expected result of that are differences in the fluidity of the membranes.

ESR measurements are often used to experimentally determine membrane fluidity. The order parameter calculated from the ESR spectrum using the fatty acid spin label 1(10,3) refers more to the static than to the dynamic components of fluidity (Stubbs and Smith 1984). Figure 1 illustrates the dependence of the order parameter on the species and on the ionic strength of the solution.
Fig. 2. The rate constants $k$ of Rb$^+$ (K$^+$)-efflux of erythrocytes from various mammalian species in solution of high ionic strength ((NaCl + KCl) = 147 mmol/l) as a function of (a) the mean number of double bonds (Wessels and Veerkamp 1973, data for cat from Deuticke 1977); $r = 0.877, p = 0.01$ (with human RBC excluded: $r = 0.908, p = 0.05$); b) the order parameter of the spin label 1(10.3) in solution of high ionic strength; $r = -0.872, p = 0.01$ (with human RBC excluded: $r = -0.885, p = 0.05$); $T = 310 K$, pH = 7.4, osmolarity = 290 mOsmol/l.

Fig. 3. The rate constants $k$ of Rb$^+$ (K$^+$)-efflux of erythrocytes from various mammalian species in solution of low ionic strength ((NaCl + KCl) = 5.7 mmol/l) as a function of (a) the mean number of double bonds (Wessels and Veerkamp 1973, data for cat from Deuticke 1977); $r = 0.772, p = 0.05$ (with human RBC excluded: $r = 0.904, p = 0.05$); b) the order parameter of the spin label 1(10.3) in solution of low ionic strength; $r = 0.932, p = 0.01$ with human RBC excluded; $T = 310 K$, pH = 7.4, osmolarity = 290 mOsmol/l.
Table 2. Rate constants \( k \) of Rb\(^{+}\)-efflux of human erythrocytes in high and low ionic strength solutions with the addition of 1 mmol/\( l \) quinine as inhibitor of Ca\(^{2+}\) induced K\(^{+}\)-efflux

<table>
<thead>
<tr>
<th>Addition</th>
<th>((\text{NaCl + KCl}) = 147 \text{ mmol/}l)</th>
<th>((\text{NaCl + KCl}) = 5.7 \text{ mmol/}l)</th>
<th>(k_{5.7/147})</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>(8.1 +/− 0.4 ) (7)</td>
<td>(63.6 +/− 4.2 ) (8)</td>
<td>7.85</td>
</tr>
<tr>
<td>Quinine</td>
<td>(7.6 +/− 0.3 ) (5)</td>
<td>(48.8 +/− 5.9 ) (10)</td>
<td>6.42</td>
</tr>
</tbody>
</table>

\( T = 310 K, \text{ pH} = 7.4, \text{ osmolarity} = 290 \text{ mOsmol/l}, \text{ means} +/− \text{ 1 S. D. The figures in brackets indicate number of experiments.} \)

Table 3. Rate constants \( k \) of Rb\(^{+}\)-efflux of human and bovine erythrocytes in the presence and absence of external potassium

<table>
<thead>
<tr>
<th>NaCl [mmol/l]</th>
<th>KCl [mmol/l]</th>
<th>(k^{*} \times 10^{4}) [1/min]</th>
<th>NaCl [mmol/l]</th>
<th>KCl [mmol/l]</th>
<th>(k^{*} \times 10^{4}) [1/min]</th>
<th>(k_{5.7/147})</th>
</tr>
</thead>
<tbody>
<tr>
<td>141.3</td>
<td>5.7</td>
<td>(8.1 +/− 0.4 ) (7)</td>
<td>0</td>
<td>5.7</td>
<td>(63.6 +/− 4.2 ) (8)</td>
<td>7.85</td>
</tr>
<tr>
<td>147</td>
<td>0</td>
<td>(5.0 +/− 1.7 ) (7)</td>
<td>5.7</td>
<td>0</td>
<td>(35.6 +/− 3.9 ) (7)</td>
<td>7.12</td>
</tr>
<tr>
<td>141.3</td>
<td>5.7</td>
<td>(7.0 +/− 0.6 ) (5)</td>
<td>0</td>
<td>5.7</td>
<td>(8.7 +/− 1.4 ) (9)</td>
<td>1.24</td>
</tr>
<tr>
<td>147</td>
<td>0</td>
<td>(6.9 +/− 1.8 ) (6)</td>
<td>5.7</td>
<td>0</td>
<td>(7.9 +/− 2.1 ) (11)</td>
<td>1.14</td>
</tr>
</tbody>
</table>

\( T = 310 K, \text{ pH} = 7.4, \text{ osmolarity} = 290 \text{ mOsmol/l}, \text{ means} +/− \text{ 1 S. D. The figures in brackets indicate number of experiments.} \)

Another useful way to estimate membrane fluidity is the analysis of lipid composition of the erythrocytes. The mean number of double bonds is considered a rough indicator of membrane fluidity (Farias et al. 1975; Deuticke 1977). In this connection it should be mentioned that the mean chain length of the phospholipids (Deuticke 1977) and the phospholipid/cholesterol ratio (Gruber and Deuticke 1973) for erythrocytes of various mammalian species are almost constant.

We attempted to correlate the rate constants of Rb\(^{+}\) (K\(^{+}\)) efflux with the “different membrane fluidities” (as estimated by different methods) in solutions of physiological and low ionic strength. The results are shown in Figs. 2a, 2b and 3a, 3b for solution of physiological and low ionic strength, respectively. The \(k_{5.7/147}\) ratio did not correlate with the different values of membrane fluidity. In each case the linear correlation coefficient of the mean values was calculated and only significant values were taken.

Two experimental series were performed to test the possible effect of Ca\(^{2+}\)-induced K\(^{+}\) efflux on the increase of the Rb\(^{+}\) (K\(^{+}\)) efflux rate constant in solution of low ionic strength compared with that measured in solution of
physiological ionic strength. The inhibitor of Ca\(^{2+}\) -induced K\(^{+}\) efflux, quinine (Armando-Hardy et al. 1975; Miner et al. 1983) was added at 1 mmol/l to both solutions. Table 2 shows the rate constants of the Rb\(^{+}\) (K\(^{+}\)) efflux for human erythrocytes. The k5.7/k147 ratio decreased only slightly after quinine treatment. As it is known that the Ca\(^{2+}\) -induced K\(^{+}\) efflux requires the presence of external potassium (Blum and Hoffman 1972; Heinz and Passow 1980) in both the standard and the low ionic strength solution all KCl was replaced by NaCl. The K\(^{+}\) concentrations of both solutions were determined by flame photometry as being below 0.01 mmol/l. The results of these experiments are given in Table 3. The rate constants for human erythrocytes were reduced in both the physiological and the low ionic strength solution, thus the k5.7/k147 ratio remained almost unchanged. In the absence of external potassium, no significant changes of the rate constants could be detected for bovine erythrocytes (Ca\(^{2+}\) -induced K\(^{+}\) efflux was not observed, see Table 1), either in the physiological or the low ionic strength solution.

**Discussion**

The results presented in this paper show that the rate constants of Rb\(^{+}\) (K\(^{+}\)) efflux in solution of physiological and low ionic strength vary widely depending on the species. Even fluxes calculated with different intracellular potassium concentrations and surface/volume ratios allowed for vary to a large extent (data not shown).

Bernhardt et al. (1984) emphasized the importance of the transmembrane, inner and outer surface potentials for the regulation of passive ion transport. Thus it seemed possible to explain the increase of the rate constant in solution of low ionic strength for human erythrocytes by introducing these values in the Goldman flux equation without assuming any change of the permeability coefficient. At least bovine erythrocytes showed electrophoretic mobility changes in response to the extracellular ionic strength similar to human erythrocytes (Erdmann et al. 1988). The transmembrane potential deduced from 36-chloride distribution was nearly the same for bovine and human erythrocytes (Bernhardt et al. 1986). Therefore these values (transmembrane potential, outer surface potential) are not likely the reason for the different fluxes measured in these two species. It is known that phosphatidylserine (the main negatively charged phospholipid) is confined to the inner leaflet in the erythrocyte membrane for man, cow and rat (Verkleij et al. 1973; van Dijck et al. 1976; Renooij et al. 1976) to nearly the same extent; this should result in a relatively high negative inner surface potential. Obviously there must be an additional and essential factor influencing the passive ion transport through the erythrocyte membrane.
The results of de Gier et al. (1968), Kirk (1977) and Lu and Chow (1982) make it probable that this factor is linked with membrane fluidity and/or the phospholipids headgroups as well as the structure of their acyl chains. It has been shown that the influence of membrane fluidity e.g. on enzyme activity is often hard to distinguish from specific lipid-protein interactions (Brasitus et al. 1986). Kuypers et al. (1984) and Bernhardt et al. (1986) could show that the content of arachidonic acid is of special significance for the potassium transport in erythrocytes. Therefore the theoretical papers of Disalvo (1985) and Rossignol et al. (1985) should be considered: these authors investigated to which extent permeation, especially of ions, may be assumed to be controlled by fluidity. At least two processes could be specified as being of importance in this respect: the diffusion from the medium into the membrane, which can be described by the partition coefficient, and the diffusion in the membrane interior.

In the present paper the proteins of the erythrocyte membrane of various mammalian species have not been considered, and this from two reasons, firstly it is still unclear whether proteins are involved in the "passive" ion transport at all; and secondly, because data from the literature considering similarities or differences in the protein patterns of various mammalian species are partly contradictory (Kobylka et al. 1972; Ralston 1975; Deuticke 1977; Fletcher et al. 1982; Whitfield et al. 1983).

As far as the influence of ionic strength on the results of the ESR measurements is concerned, the found values of the order parameters for all species investigated were smaller in low ionic strength solution than in that of physiological ionic strength. Herrmann and Müller (1986) could demonstrate that these differences reflect true alterations of the membrane structure rather than being due to the method or the spin label used. The observation that the membrane fluidity increases from the extracellular surface toward the membrane centre (Ogiso et al. 1981; Herrmann and Müller 1986) make us think that the order parameter calculated from the spectra of the label I(10.3) might reflect the properties of a membrane region decisive for ion transport. The mechanism underlying the changes of membrane fluidity remains unclear. Both the transmembrane potential and the outer surface potential, which are simultaneously changed upon decreasing the ionic strength of the solution (replacement of NaCl by sucrose), might well influence membrane fluidity. This may be explained by a change of the packing density of membrane lipids, by a direct effect of the electric field on protein conformation, and/or by an indirect effect on the interaction between proteins and lipids mediated by the glyocalyx. A protein change-induced phospholipid flip-flop and the resulting alteration of the phospholipid asymmetry might be an additional possibility. Also, changes in the area between different lipid domains should be considered (Cruzeiro—Hansson and Mouritsen 1988). Based upon the two values used in the present paper to
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characterize membrane fluidity relatively high and almost identical correlation coefficients (see Fig. 2) of the \( \text{Rb}^+ (K^+) \) efflux rate constants were obtained for physiological ionic strength solution and the above given values. For the rate constant in low ionic strength solution it should be mentioned that comparable correlation coefficients for the order parameter and for the mean number of double bonds could only be obtained if the values for human erythrocytes were excluded (see Fig. 3). The mean number of double bonds and the rate constant in low ionic strength solution, however also gave a significant correlation with the values for human erythrocytes considered. Essentially, the value of the correlation coefficient was then lower. It should also be considered that the values of the mean number of double bonds (taken from the literature) have been determined for erythrocytes suspended in high ionic strength solutions. It can be concluded that membrane fluidity plays an important role in the regulation of the passive ion transport through the membrane of various erythrocytes.

The findings of this paper can be summarized as follows:
(i) The passive \( \text{Rb}^+ \) transport in erythrocytes of all the species investigated, with the exception of human erythrocytes, was highly influenced by membrane fluidity, regardless of the method used to estimate it and regardless of the conditions under which the efflux was measured (see also Erdmann et al. 1987).
(ii) A different mechanism of passive ion transport or an additional separately low ionic strength stimulated mechanism could be supposed for human erythrocytes. As this additional transport mechanism the \( \text{Na}^+, \text{K}^+, \text{Cl}^- \) cotransport is considered. This mechanism is of a significant extent only in human erythrocytes (Ellory et al. 1982). Furosemide, an inhibitor of the cotransport mechanism reduced the rate constant of \( \text{Rb}^+ \) efflux from human erythrocytes by 50% both in solution of high and of low ionic strength (Bernhardt et al. 1987), whereas no changes were recorded with cow and horse erythrocytes (data not shown). Using these values for human erythrocytes, the following correlation coefficients are obtained: Figure 2a: \( r = 0.700 \) (insignificant), 2b: \( r = -0.885 \) \( (p = 0.01) \), 3a: \( r = 0.916 \) \( (p = 0.01) \), 3b: \( r = -0.778 \) \( (p = 0.05) \).

This stresses the possible role of membrane fluidity in the regulation of passive potassium fluxes also in human erythrocytes.

References

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Final version accepted June 20, 1990