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Influence of Surface Charge and Transmembrane Potential on Rubidium-86 Efflux of Human Red Blood Cells

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Summary. The dependence of the rate constant of Rb^+ efflux on extracellular cation concentration was measured. At low ionic strengths Rb^+ efflux increased strongly. Permeability coefficients were calculated from the rate constants measured, using the Goldman flux equation, with and without making allowance for surface potentials. Only when allowance was made for surface potentials and the associated differences beween ion concentrations in the bulk solutions and at the membrane surface, the permeability coefficient remained constant. Best agreement between experimental data and theoretically calculated values was obtained when an interior surface potential of -110 mV was assumed.

When the surface charge of erythrocytes is reduced by neuraminidase, the rate constants for Rb^+ efflux decreased, indicating a significant influence of surface potential.

Key Words transmembrane potential · surface potential · red blood cell · permeability · Rb^+ efflux

Introduction

As indicated by various findings, the passive K^+ efflux of human erythrocytes can be influenced by a number of factors. One possibility is the effect, described by Davson (1939), that erythrocytes in isotonic solutions of low ionic strength increasingly lose potassium. This effect which was confirmed by a number of other authors (Wilbrandt, 1940; Wilbrandt & Schatzmann, 1960; LaCelle & Rothstein, 1966) was interpreted by Donlon and Rothstein (1969) as a change of K^+ permeability of erythrocytes dependent on transmembrane potential.

Our investigations suggest an additional influence of surface potential in the case of changed K^+ efflux of human erythrocytes in solutions of low ionic strength (Bernhardt, Borning & Glaser, 1982). The role of surface potential of cells which is also a function of exterior ionic strength was not allowed for in the studies of Donlon and Rothstein (1969). But Wilbrandt (1940) and Wilbrandt and Schatzmann (1960) already qualitatively discussed the role of the membrane surface potentials for the explanation of the increased K^+ efflux at low ionic strength. Now it is the aim of this paper to investigate this possibility quantitatively.

Materials and Methods

1. Erythrocyte Preparation

Human erythrocytes of the 0 Rh^+ group from 2-day-old whole-blood citrate preserves were used. The erythrocytes were separated from blood plasma by centrifugation (2,000 × g, 8 min) and subsequently washed two times (2,000 × g, 8 min) in the suspending solution (mM: NaCl 141.3, KCl 5.7, glucose 5.0, Na_2HPO_4/NaH_2PO_4 5.8, pH 7.4). To establish a steady state at a hematocrit of HC = 32%, the cells were incubated at 310 °K for 1 hr.

Working temperature was 310 K in efflux experiments and 293 to 295 K in chloride distribution experiments. The pH was 7.4 in all cases. In solutions with reduced ionic strength, osmolarity was adjusted to 290 mOsm by adding sucrose. In all solutions used the KCl concentration was 5.7 mM.

2. Incubation with Neuraminidase

After separation of blood plasma 1.3 ml erythrocyte suspension (HC = 45%) were incubated with 1.7 U α-neuraminidase from Vibrio cholerae (SERVA) at 310 K for 1 hr, one unit being the amount of enzyme required to release 1 μg of N-acetyl-neuraminic acid from human α_1-acid glycoprotein at 310 K within 1 min. Then, the erythrocytes were removed by centrifugation at 2,000 × g and 277 K and washed twice under the same conditions for 8 min (Lerche, Hessel & Donath, 1980). Then, they were further treated as described above beginning with the one-hour incubation.

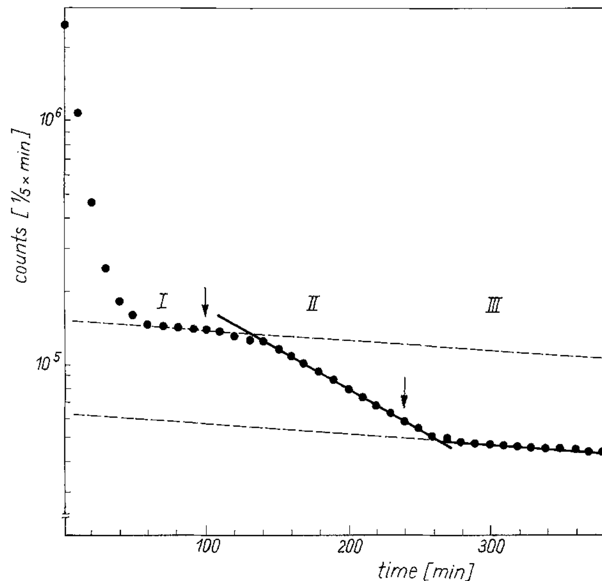

Fig. 1. Radioactivity decrease of Rb-86-loaded erythrocytes at sudden changes of flushing solution (↓). *I:* Initial radioactivity decrease using the suspending solution (mM: NaCl 141.3, KCl 5.7, glucose 5.0, Na_2HPO_4/NaH_2PO_4 5.8, pH 7.4) as flushing solution. *II:* Low ionic strength solution (mM: NaCl 0, KCl 5.7, glucose 5.0, Na_2HPO_4/NaH_2PO_4 5.8, pH 7.4) as flushing solution. *III:* Flushing solution as in *I*

3. Rb-86 Efflux Measurement

1.8 to 2.2 MBq/ml Rb-86-Cl (Rb^+ as indicator for K^+) were added to the erythrocyte suspension and an further one-hour incubation at 310 K followed. The erythrocytes were then introduced into a diffusion chamber. This chamber (vol 0.1 ml) is closed by a membrane filter (SARTORIUS, pore size 3 µm) placed in the measuring chamber and continuously flushed with nonradioactive solution. The flow rate of this solution was 5 ml/min. Under this condition no detectable changes of the pH and the ionic concentrations occurred in the flushing part (vol 20 ml) of the measuring chamber. To prevent sedimentation, the diffusion chamber was mounted on a slowly rotating horizontal axle (12 rpm). The radioactivity in the measuring chamber was measured continuously by scintillation counting and thus the time course of radioactivity release could be followed. As the rate constant of the ^{86}Rb flux from the extracellular solution into the flushing solution is very high (0.12 min^{-1}), practically, the radioactivity release from the erythrocytes is measured. Only in the beginning of the experiment the fast compartment can be detected (*cf.* Fig. 1). In the course of the experiment the measuring chamber was successively flushed with two different solutions. Within the first 100 min the suspending solution, described above was used. This solution was then replaced by a solution of the desired composition. To check the reversibility of the change of the rate constant observed, in a number of experiments the initial solution (suspending solution) was finally used again. The rate constant of Rb^+ efflux was directly determined by graphic analysis. One typical experiment is shown in Fig. 1.

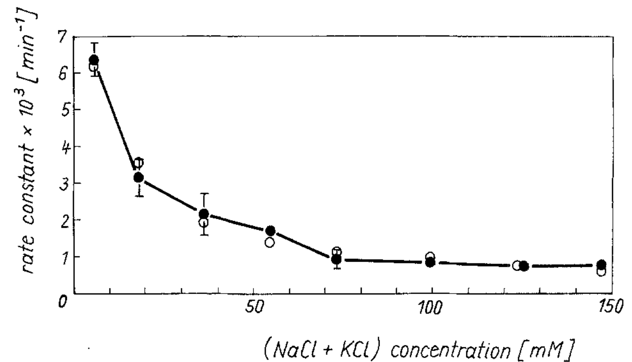

Fig. 2. Dependence of rate constant of Rb^+ efflux on extracellular (NaCl + KCl) concentration. (pH = 7.4; T = 310 K; osmolarity = 290 mOsm). ●, experimental values; ○, values calculated (Eqs. 5, 6) assuming a permeability coefficient of 1.30×10^{-10} m·sec^{-1} and an interior surface potential of −113.7 mV. Vertical bars indicate ±1 SD.

4. Cl-36 Distribution Measurement

The erythrocytes prepared according to section 1 were centrifuged at 2,000 × g for 8 min. One ml sediment was added to 9.9 ml Cl-36 (sodium salt) labeled solutions (1 kBq/ml) of different Cl^- concentrations as described in section 1 and continuously stirred. After different time intervals (3 to 60 min) 1 ml suspension was centrifuged at 1,000 × g for 90 sec. 0.1 ml supernatant was added to 8 ml of scintillation liquid containing 100 g naphthalene, 4 g 2,5 diphenyloxazole, 0.2 g *p-bis* 2-(5-phenyloxazolyl)-benzene per liter dioxane. The radioactivity was measured in the P-32 channel of a liquid scintillation counter (Ultrabeta 1210, LKB-Wallac). This way four different blood samples were measured 3 times and the Cl-36 distribution was determined, taking into account the initial total Cl-36 content of the solutions measured prior to the addition of the erythrocytes. The Cl^- concentration was both corrected for the addition of the 2.37 M NaCl-36 solution and for the trapped NaCl of the sediment. The cell water content was assumed to be 0.7 ml/ml cells.

Results and Discussion

The effect of increased K^+ efflux in isotonic solutions of low ionic strength, described by various authors (Wilbrandt, 1940; Wilbrandt & Schatzmann, 1960; LaCelle & Rothstein, 1966; Donlon & Rothstein, 1969), could be confirmed also by us. The change of the rate constant of Rb^+ efflux in its dependence on extracellular (NaCl + KCl) concentration is represented in Fig. 2. Previously, we showed that the total Na^+ efflux remains practically constant if the extracellular NaCl concentration decreases. However, the ouabain-insensitive Na^+ efflux increases significantly, but this increase is compensated by a corresponding decrease of the ouabain-sensitive Na^+ efflux (Bernhardt & Glaser, 1982).

When the extracellular NaCl concentration is varied, the transmembrane potential changes and, in addition, due to the altered ionic strength, the surface potential changes as well. Rb^+ permeability can be calculated by the Goldman flux equation (Goldman, 1943):

$$J_{io} = \frac{z \cdot P \cdot F \cdot \Delta\psi}{R \cdot T} \cdot \frac{c_i \cdot \exp(z \cdot F \cdot \Delta\psi / R \cdot T)}{\exp(z \cdot F \cdot \Delta\psi / R \cdot T) - 1} \quad (1)$$

where J_{io} = efflux of an ionic species, P = permeability coefficient, c_i = intracellular concentration of the respective ion and $\Delta\psi$ = transmembrane potential. z, F, R, T are the usual charge number, Faraday constant, gas constant and temperature.

Efflux J_{io} can be determined from the measured rate constant k by means of the relationship:

$$J_{io} = k \cdot c_i \cdot V/A \quad (2)$$

where V = cell volume and A = surface of cells.

The cell volume of erythrocytes changes as a function of extracellular chloride concentration. In accordance with a model published earlier, both volume change and transmembrane potential can be calculated for the respective extracellular chloride concentration (Brumen, Glaser & Svetina, 1979; Glaser, 1979, 1982); compare equations CI–CIII and the additional equations in Glaser (1982). The basis of this theory is that a chloride equilibrium is assumed as the net chloride permeability exceeds the cation permeabilities by two orders of magnitude (Hunter, 1977; Knauf, Fuhrmann, Rothstein & Rothstein, 1977).

Additionally, we measured the chloride distribution in solutions of different Cl^- concentration using Cl-36. It was found that the chloride distribution during the course of the experiment (3 to 60 min) does not change. This is consistent with the assumption that even at low ionic strength the cation permeability is considerably smaller than the chloride permeability. Thus it follows that with the Nernst equation from the chloride distribution measured the transmembrane potential can be calculated (Donlon & Rothstein, 1969). Figure 3 compares the calculated transmembrane potentials according to Glaser (1982) and the experimentally determined values in dependence on the external (NaCl + KCl) concentration. A reasonable coincidence is observed.

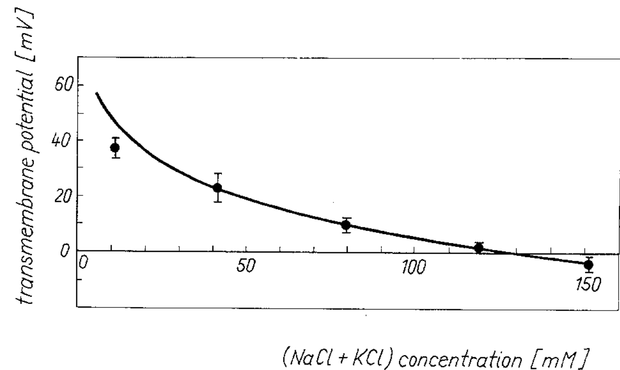

Fig. 3. Dependence of the transmembrane potential on the extracellular (NaCl + KCl) concentration. (pH = 7.4; osmolarity = 290 mOsm). ●, transmembrane potentials calculated by using the measured Cl-36 distribution assuming Nernst equilibrium; solid curve: calculated values according to Glaser (1982). Vertical bars indicate ±1 SD.

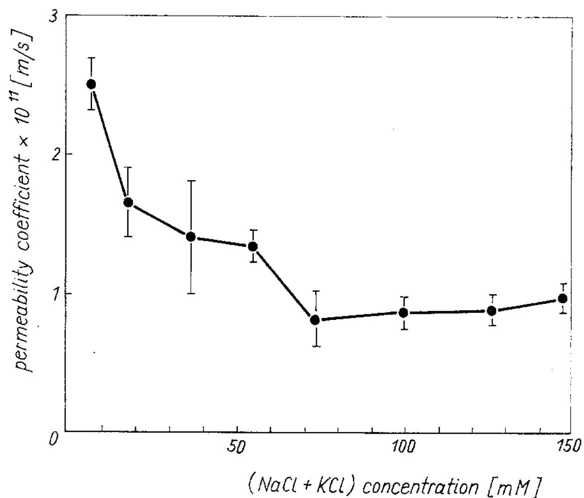

Fig. 4. Dependence of Rb^+ permeability coefficient (determined by Eq. (1) assuming an erythrocyte surface of 137 μm^2 on extracellualr (NaCl + KCl) concentration. (pH = 7.4; T = 310 K; osmolarity = 290 mOsm). Vertical bars indicate ±1 SD.

The permeability coefficients determined from the measured rate constant (Fig. 2) by Eq. (1) are shown in Fig. 4, assuming an erythrocyte surface of 137 μm^2 (Jay, 1975). It is evident that the permeability increases as the external chloride concentration falls. Such behavior suggests that, at changed transmembrane potential, the ion flux changes beyond that expected due to the change in electrochemical driving force. Such an interpretation was given by Donlon and Rothstein (1969).

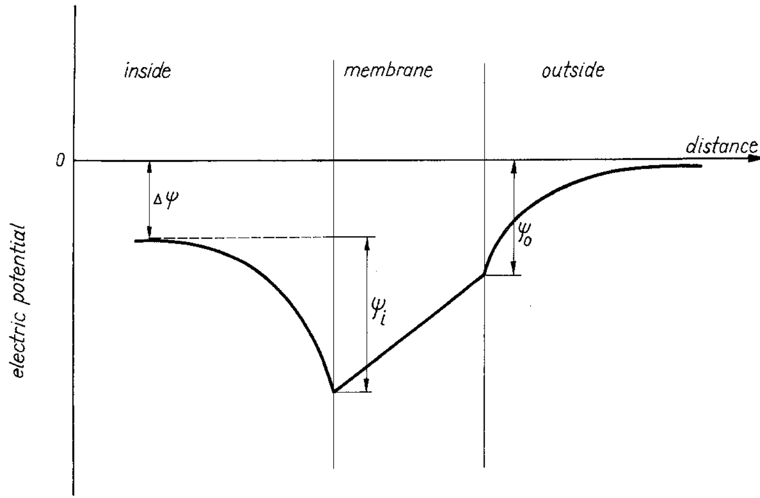

Fig. 5. Hypothetical potential profile across membrane. $\Delta\psi$, transmembrane potential; ψ_0, outer surface potential; ψ_i, inner surface potential

Here it seems necessary to point out that the equation derived by Goldman (1943) does not allow for surface potentials. McLaughlin (1977) discussed a possible influence of electric potential difference between membrane surface and free solution on ion transport. According to him, at modified surface potential, a change in the ion transport rate would be expected due to the change of ion concentration at the immediate membrane surface. Now the aim is to prove this idea quantitatively comparing the experimental results with the theoretical predictions. If then it is possible to explain the increase of the Rb^+ efflux in solutions of low ionic strengths without assuming additionally Rb^+ permeability change, the chloride equilibrium treatment for the membrane potential calculation is justified even when the rate constant of Rb^+ efflux is increased.

Assuming the existence of surface charges at both the interior and exterior membrane surface, the potential profile across the membrane can be illustrated as shown in Fig. 5. The changed ion concentrations at the membrane surface can be calculated from the ion concentrations of the free solution using the Boltzmann relationship:

$$\begin{aligned} c_{i,o} &= c_i \cdot \exp(z \cdot F \cdot (-\psi_i)/R \cdot T) \\ c_{o,o} &= c_o \cdot \exp(z \cdot F \cdot (-\psi_o)/R \cdot T) \end{aligned} \tag{3}$$

where $c_{i,o}$ and $c_{o,o}$ represent ion concentrations at the membrane surface, c_i and c_o are ion concentrations of the free solution and ψ_i and ψ_o, respectively are the interior and exterior surface potential as illustrated in Fig. 5.

Allowing for these relationships, the following expression was derived for an efflux of monovalent cations, in analogy with the Goldman flux equation, by Mackey (1975) and Goulden (1976):

$$J_{io} = \frac{P \cdot F(\Delta\psi + \psi_i - \psi_o)}{R \cdot T} \cdot \frac{c_i \cdot \exp(F(\Delta\psi)/R \cdot T)}{\exp(F(\Delta\psi + \psi_i)/R \cdot T) - \exp(F(\psi_o)/R \cdot T)}. \tag{4}$$

Symbols are the same as in Eq. (1). In addition, the interior surface potential (ψ_i) and the exterior surface potential (ψ_o) are included.

Also, in the flux equation (Eq. 2), the ion concentration at the membrane surface ($c_{i,o}$) has to be used instead of the concentration in the bulk phase. Thus it follows:

$$J_{io} = k \cdot c_{i,o} \cdot V/A. \tag{5}$$

Equation (4), including Eq. (3), can be written in the following form:

$$J_{io} = \frac{P \cdot F(\Delta\psi + \psi_i - \psi_o)}{R \cdot T} \cdot \frac{c_{i,o} \cdot \exp(F(\Delta\psi + \psi_i)/R \cdot T)}{\exp(F(\Delta\psi + \psi_i)/R \cdot T) - \exp(F(\psi_o)/R \cdot T)}. \tag{6}$$

The exterior surface potential can be calculated either in accordance with the classical concept of Gouy and Chapman or using the theory of Donath and Pastushenko (1979) who proceed from a spatial distribution of fixed charges on the cell surface in the glycocalyx. We assumed

Table 1. Measured rate constant (k) of Rb^+ efflux, calculated transmembrane potentials ($\Delta\psi$) and volume/surface relationships (V/A) according to Glaser (1979, 1982), exterior surface potentials (ψ_0) according to Gouy and Chapman (a) and according to Donath and Pastushenko (1979) (b) as functions of extracellular (NaCl + KCl) concentration

(NaCl + KCl) concentration (mM)	$k \cdot 10^3$ (min^{-1})	$\Delta\psi$ (mV)	$V/A \cdot 10^6$ (m)	ψ_0 (a) (mV)	ψ_0 (b) (mV)
5.7	6.36 ± 0.42	52.3	0.54	−59.42	−21.11
18.3	3.10 ± 0.50	34.7	0.57	−47.26	−18.86
36.7	2.10 ± 0.60	22.0	0.59	−38.13	−9.21
54.7	1.71 ± 0.14	14.1	0.61	−32.92	−6.92
73.5	0.90 ± 0.22	8.0	0.62	−29.26	−5.49
99.7	0.83 ± 0.11	1.6	0.63	−25.73	−4.25
125.7	0.76 ± 0.09	−3.4	0.64	−23.25	−3.48
147.0	0.81 ± 0.09	−6.8	0.64	−21.68	−3.03

pH = 7.4; T = 310 K; osmolarity = 290 mOsm. The values are given as mean ± 1 SD.

2×10^7 negative charges per cell and converted this value into surface charge density using an area of 137 µm² for the Gouy-Chapman model and into a space charge density assuming a glycocalyx thickness of 5.5 nm, respectively (Donath & Lerche, 1980).

Table 1 lists the rate constants measured as function of extracellular (NaCl + KCl) concentration, the transmembrane potentials and the volume/surface relations calculated according to Glaser (1979, 1982), and the surface potentials calculated according to both Gouy and Chapman and Donath and Pastushenko (1979), respectively. The ionic strength of the buffer system (5.8 mM Na_2HPO_4/NaH_2PO_4, pH 7.4) was assumed to be 16 mM for this calculation. Estimates of the permeability coefficient and interior surface potential were made by fitting the experimental data to Eq. (6) using a gradient method. Two different ways were considered with respect to exterior surface potential. Firstly, using the model of Donath and Pastushenko (1979) the best agreement was obtained at a permeability coefficient of 1.30×10^{-10} m·sec^{-1} and an interior surface potential of −113.7 mV. Secondly, on the basis of the exterior surface potential according to Gouy and Chapman the best agreement could be obtained at a permeability coefficient of 4.12×10^{-11} m·sec^{-1} and an interior surface potential of −107.4 mV. The rate constants calculated by means of a permeability coefficient of

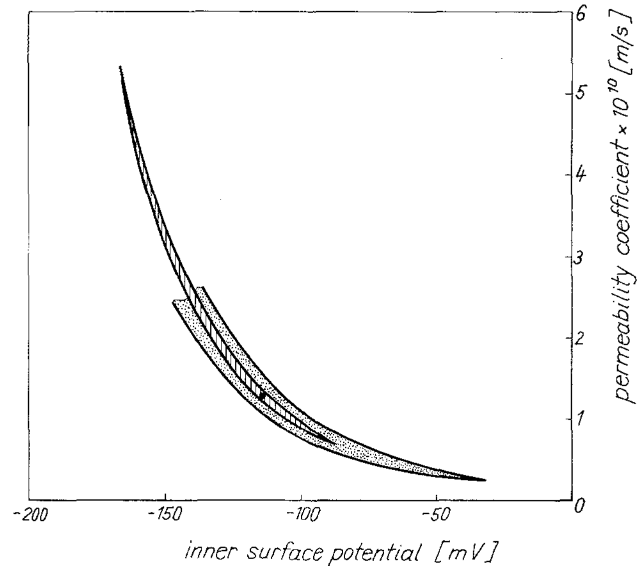

Fig. 6. Correlation between the fitted parameters permeability coefficient and inner surface potential. Dotted area – range of parameters where the deviation D (Eq. 7) is less than 10%; hatched area – range of parameters where the deviation D (Eq. 7) is less than 2.5%; solid point – absolute minimum of deviation, values given in text

1.30×10^{-10} m·sec^{-1} and an interior surface potential of −113.7 mV are also shown in Fig. 2.

However, critical analysis shows that there is a strong correlation between the fitted permeability coefficient and inner surface potential. This means that it is not possible to give standard deviations for the estimated parameters. Figure 6 shows, therefore, the range of parameters where the deviation D of the theoretical curve from the experimental one is less than 10 or 2.5%, respectively. Both curves refer to the first model of the outer surface potential (Donath & Pastushenko, 1979). D is defined as follows: let us denote by k_i the measured rate constants, by $\bar{k}$ the average rate constant and by k_i^t the calculated rate constant according to assumed values of P and ψ_i. Then D can be expressed by:

$$D = \frac{\sum_i (k_i - k_i^t)^2}{\sum_i (k_i - \bar{k})^2} \quad (7)$$

where the summation index i runs over the experimentally determined rate constants.

From the analysis (*compare* Fig. 6) we conclude that the provided experiment is not sufficient to quantitatively determine permeability coefficient and interior surface potential inde-

pendently. However, qualitatively, it can be stated that a relatively high negative interior surface potential explains the fact that the observed rate constant increases at low ionic strengths. From data on the lipid composition of the human erythrocyte membrane we know that almost all phosphatidylserine is confined to the inner leaflet of the bilayer at a surface concentration of about 20% (Zwaal, Roelofsen, Comfurius & van Deenen, 1975; Marchesi, Furthmayr & Tomita, 1976). Papahadjopoulos (1968) has measured the electrophoretic mobility of phosphatidylcholine vesicles with 20% of phosphatidylserine in 145 mM NaCl. From this data a ζ potential of -30 mV can be estimated. On the other hand Eisenberg, Gresalfi, Riccio and McLaughlin (1979) have determined for pure phosphatidylserine bilayers a surface potential of about -100 mV. Thus it seems to be reasonable to expect an interior surface potential value within the range -30 to -100 mV. If we allow 10% deviation the fitted values of the interior surface potential (Fig. 6) will cover a range from about -30 mV to very high negative surface potentials. The conclusion is that an interior surface potential in the range -30 to -100 mV can explain the dependence of the rate constant on exterior ionic strength without assuming additional nonelectrochemical effects.

These results indicate the importance of surface potentials in the control of ion transport at biological membranes. This statement can be further supported by the following experimental results.

We reduced the surface charge of erythrocytes by enzymatic degradation with neuraminidase. From electrophoretic mobility measurements of erythrocytes a charge reduction of about 90% was inferred (Donath & Lerche, 1980). Table 2 presents the measured rate constant k and the calculated rate constant k^t according to Eqs. (5) and (6) with and without neuraminidase treatment in solutions of different (NaCl+KCl) concentration. It is assumed that neuraminidase treatment does not influence either the Rb^+ permeability or the transmembrane potential directly. It can be seen that the rate constant of the Rb^+ efflux after charge reduction by neuraminidase in solutions of low ionic strength decreases significantly while in solutions of high ionic strength no significant change is observed. This observation is consistent with the theoretical prediction calculated in Table 2. This proves additionally the idea of the dependence of Rb-86 efflux on the electric potential difference between the two membrane surfaces.

Table 2. Comparison of measured (k) and calculated (k^t) rate constants (Eqs. 5, 6) of the Rb^+ efflux in solutions of low and high (NaCl+KCl) concentration with and without neuraminidase treatment[a]

(NaCl +KCl) concentration (mM)	Charge reduction (%)	$k \cdot 10^3$ (min^{-1})	$k^t \cdot 10^3$ (min^{-1})	ψ_0 (mV)	$\Delta\psi$ (mV)
5.7	0	6.36 ± 0.42	6.19	-21.1	52.3
5.7	90	2.53 ± 0.52	3.90	-2.1	52.3
147.0	0	0.81 ± 0.09	0.79	-3.0	-6.8
147.0	90	0.77 ± 0.11	0.73	-0.3	-6.8

[a] Outer surface potential ψ_0 calculated according to Donath and Pastushenko (1979), transmembrane potential $\Delta\psi$, compare Table 1. Permeability coefficient and inner surface potential are assumed to be 1.30×10^{-10} m·sec^{-1} and -113.7 mV, respectively. pH=7.4; T=310 °K; osmolarity=290 mOsm. The values are given as mean ±1 SD.

Also the effect described by Wilbrandt (1940) and Bolingbroke and Maizels (1959) – that in solutions of low ionic strength a drastic reduction of K^+ efflux can be induced by adding small amounts of Ca^{2+} – indicates the importance of surface potentials in the control of passive ion transport of human erythrocytes. Thus, to describe the passive Rb^+ efflux, the true electric potential difference between the inner and outer membrane surface has to be considered. Therefore both inner and outer surface potential should be introduced into the Goldman flux equation. Both surface potentials as well as transmembrane potential can influence the passive ion transport of human erythrocytes. The extended Goldman equation (Eq. 6) can be used to describe quantitatively the effect of increasing Rb^+ efflux at low ionic strengths.

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