The Monovalent Cation “Leak” Transport in Human Erythrocytes: An Electroneutral Exchange Process

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ABSTRACT The mechanism of the “ground permeability” of the human erythrocyte membrane for K⁺ and Na⁺ was investigated with respect to a possible involvement of a previously unidentified specific transport pathway, because earlier studies showed that it cannot be explained on the basis of simple electrodiffusion. In particular, we analyzed and described the increase in the (ouabain + bumetanide + EGTA)-insensitive unidirectional K⁺ and Na⁺ influxes as well as effluxes (defined as “leak” fluxes) observed in erythrocytes suspended in low-ionic-strength media. Using a carrier-type model and taking into account the influence of the ionic strength on the outer surface potential according to the Gouy-Chapman theory (i.e., the ion concentration near the membrane surface), we are able to describe the altered “leak” fluxes as an electroneutral process. In addition, we can show indirectly that this electroneutral flux is due to an exchange of monovalent cations with protons. This pathway is different from the amiloride-sensitive Na⁺/H⁺ exchanger present in the human red blood cell membrane and can be characterized as a K⁺(Na⁺)/H⁺ exchanger.

INTRODUCTION

Although it has been investigated for many years, the mechanism of the “ground-state leak” of ions through nonexcitable cell membranes remains obscure. In practice, this “ground-state leak” (also referred to as “residual transport”) is defined as membrane transport under conditions where all known specific transport pathways (pumps, channels, carriers) are inhibited. For such studies the erythrocyte is often used as a simple model cell because the specific ion transporters in this cell type are well characterized (see, e.g., Lew and Bookchin, 1986; Bernhardt et al., 1988; Deuticke et al., 1990). This is especially true for the K⁺ transport pathways. The leak K⁺ transport across the human erythrocyte membrane is defined as (ouabain, [bumetanide or furosemide], and EGTA)-insensitive, because under these conditions the Na⁺/K⁺ pump, the Na⁺K⁺/2Cl⁻ cotransport, and the Ca²⁺-activated K⁺ channel, respectively, are inhibited. The volume-sensitive K⁺/Cl⁻ cotransport is silent in mature human red blood cells and can be activated under specific conditions only (Hall and Ellory, 1986). The study of “leak” Na⁺ fluxes across the erythrocyte membrane is more complicated because of the greater variety of specific transport systems for Na⁺ compared to K⁺ (Bernhardt et al., 1988).

The processes underlying the “leak” K⁺ and Na⁺ fluxes across the erythrocyte membrane are still a matter of debate, although various maneuvers are known to increase them, including lowering the extracellular NaCl concentration (with sucrose or lactose replacement to keep the osmolarity constant) (Davson, 1939; Wilbrandt, 1940; Wilbrandt and Schatzmann, 1960; Carolin and Maizels, 1965; LaCelle and Rothstein, 1966; Donlon and Rothstein, 1969; Bernhardt, 1981; Bernhardt et al., 1984, 1991; Jones and Knauf, 1985). Under low-ionic-strength (LIS) conditions, “leak” K⁺ and Na⁺ transport (both unidirectional influxes and effluxes as well as net effluxes) are markedly enhanced in human erythrocytes (Denner et al., 1993). In addition, the replacement of NaCl in the extracellular solution by Na gluconate or Na-glucuronate does not result in a significant change of the “leak” K⁺ influx, although such conditions lead to the same change in transmembrane potential as compared with solutions where NaCl is replaced by sucrose. From these results it was concluded that the “leak” K⁺ and Na⁺ transport cannot be explained as simple electrodiffusion (Bernhardt et al., 1988, 1991).

Our previous explanation for the increase in the “leak” K⁺ and Na⁺ fluxes under LIS conditions was based on a carrier mechanism (Denner et al., 1993). Although this approach was partly successful, we had to propose the existence of an arbitrary modifier site on the transporter to explain the experimental data. The aim of the present paper was to extend the idea of a carrier mechanism for the explanation of the “leak” K⁺ and Na⁺ flux on the basis of additional experimental data. We now assume that the “leak” transport of K⁺ and Na⁺ is realized by an electroneutral K⁺(Na⁺)/H⁺ antiporter depending on the substrate (cation) concentration near the membrane surface, and hence the membrane surface potential. Part of the presented work has been published in abstract form (Richter et al., 1996).

MATERIALS AND METHODS

Experimental

Blood

Stored bank blood from healthy donors was used for the experiments. Red blood cells were separated by centrifugation for 8 min at 1500 × g at room
temperature. Plasma and buffy coat were aspirated, and the cells were washed three times with physiological (high ionic strength, HIS) solution containing (mM): 145 NaCl, 10 glucose, 5.8 NaH₂PO₄/Na₂HPO₄, pH 7.4 at room temperature.

In experiments where NaCl in HIS solution was replaced by Na-gluconate, Na-tartrate, or (Na-tartrate + sucrose), or partly or completely by N-methyl-d-glucamine-chloride (NMDG-Cl), cells were washed twice in the NaCl-containing HIS solution. The final wash was carried out in a HIS solution containing (mM): 145 NaCl (Na-gluconate), or 107 Na-tartrate, or 55 Na-tartrate plus 120 sucrose, or 145 = X NMDG-Cl (X being the NaCl concentration, 0–145 mM or 0–80 mM in the presence of 0 mM or 65 mM KCl, respectively), 5.8 NaH₂PO₄/Na₂HPO₄, pH 7.4 at room temperature. In the series of experiments in which NaCl was replaced gradually by NMDG-Cl, the total (NaCl + NMDG-Cl) concentration was increased to 150 mM (compensated by a reduction of 7.5 mM KCl normally used for the flux (see below) to 2.5 mM KCl). When the flux measurements were carried out in LIS solution, in the final wash the cells were suspended in a solution of the following composition (mM): 250 sucrose, 10 glucose, 5.8 NaH₂PO₄/Na₂HPO₄, pH 7.4 at room temperature. In one series of experiments, 145 mM NMDG-Cl of the HIS solution was partly or completely replaced with sucrose. Also in this case, the final wash was carried out in the corresponding solution.

For proton inhibition experiments, in addition to phosphate-buffered solutions, media that did not contain phosphate buffer were used (see also Flux Measurements, below). In another set of experiments, cells were washed two times at room temperature with a HIS solution containing (mM): 145 choline-Cl, 10 glucose, 10 morpholinoethane sulfonic acid (MES), 10 Tris(hydroxymethyl)aminomethane (TRIS), pH 7.2. For this set of experiments, the final wash was carried out in solutions containing (mM): 145, 50, 20, or 0 choline-Cl; 0, 164, 215.5, or 250 sucrose; 10 glucose; 10 MES; 10 TRIS. The pH of each solution was adjusted to 7.2, 7.5, 8.0, 8.5, or 9.0 with choline-Cl and continuously measured with a pH electrode placed in parallel to the flux. Such conditions were chosen to avoid Na⁺ flux in the extracellular solution and to measure the “leak” K⁺ influx at higher pH (compared to the pH range of the phosphate-buffered solutions). In addition, the ionic strength of the MES/TRIS buffer system does not change considerably in the pH interval used.

To investigate the influence of different trans (intracellular) proton concentrations on the “leak” K⁺ influx at the same extracellular pH, red blood cells were washed twice at room temperature in an unbuffered NaCl-containing HIS solution at room temperature. The pH of the cell suspension (hematocrit ≈ 0.05) was adjusted with 0.1 N HCl and 0.1 N NaOH to 6.0 and 8.0, respectively. After the addition of dipyridamole (50 μM) to inhibit the anion exchanger, cells were centrifuged and the supernatant was removed. Then the erythrocytes were washed two times in a HIS solution containing (mM): 145 NaCl, 10 glucose, 0.1 ouabain, 0.1 bumetanide, 0.1 EGTA, 0.05 dipyridamole, 10 MES, 10 TRIS, pH 7.2. The cell suspensions were divided for flux and parallel extracellular pH measurements. In addition, the intracellular pH was determined from samples taken at the beginning and the end of the incubation period (flux period). After the erythrocytes were washed in the same solution as before (but without MES/TRIS), packed cells were hemolyzed by the addition of distilled water. pH was measured with a conventional glass electrode.

To prevent an increase in the cell volume, the solutions for the experiments of reduced internal pH (HCl adjusted) contained, in addition to the composition given above, 30 mM sucrose (Lew and Bookchin, 1986). All other solutions (except the solutions that did not contain 5.8 mM NaH₂PO₄/Na₂HPO₄ or 10 mM MES and 10 mM TRIS) had the same osmolarity (300 mosM, measured with a vapor pressure osmometer).

Flux measurements

The methods of flux measurements have been published elsewhere (Bernhardt et al., 1991; Denner et al., 1993). ⁸⁶Rb was used as a tracer for K⁺. Usually, for K⁺ influx measurements, ⁸⁶Rb (in KCl) was added to the flux tubes to start the flux. The final K⁺ concentration in the flux solutions was usually 7.5 mM. Only in experiments in which NaCl was gradually replaced by NMDG-Cl was the final KCI concentration 2.5, 7.5, or 72.5 mM. Fluxes were measured at 37°C. The compositions of the flux media are given in the figure legends.

In one set of experiments at different pH of the extracellular solution during the flux measurement (see proton inhibition), 7.5 mM KCI was gradually replaced by 7.5 mM KHCO₃ − X (X = KCl, 0–7.5 mM). This leads to different extracellular H⁺ concentrations, which were measured with a pH electrode in parallel to the flux. These experiments were carried out in the phosphate-buffered HIS and LIS solution (see above), as well as in those solutions without phosphate buffer. The absence of 5.8 mM NaH₂PO₄/Na₂HPO₄ in LIS media leads to a further reduction in the ionic strength of the extracellular solution.

To investigate the influence of charged amphiphilic molecules that intercalate into the membrane on the “leak” K⁺ influx, the flux media contained various concentrations (0–100 μM) of sodium dodecyl sulfate (SDS) or dodecytrimethylammonium bromide (DDDTMA). These amphiphiles have an identical alkyl chain length (12 carbon atoms) and carry a negative (SDS) or a positive (DDDTMA) charge. For these experiments the erythrocytes were preincubated in the presence of the substances at 37°C for 5 min.

Reagents

Inorganic salts, sucrose, and glucose were analytical grade. Ouabain, bumetanide, EGTA, Na-tartrate, NMDG, SDS, DDTMA, choline-Cl, and choline-OH were obtained from Sigma Chemical Company (St. Louis, MO). Na-gluconate was purchased from BDH Chemicals (Poole, England). MES was obtained from SERVA GmbH (Heidelberg, Germany), TRIS was purchased from Fluka Chemie AG (Buchs, Switzerland), and dipyridamole was obtained from Research Biochemicals International (Natick, MA). ⁸⁶Rb (in RbCl) was produced by Amersham International (Amer- sham, England). NMDG was titrated with HCl to pH 7.4 to obtain NMDG-Cl.

Theoretical background

According to the Gouy-Chapman theory, one can calculate the surface potential of a biological membrane and the potential profile near the surface. In the framework of this theory, it is assumed that the surface charges are continuously smeared at the membrane surface. The surface charge density (σ) and the ionic strength (I) are related (Gouy, 1910; Chapman, 1913):

$$\sigma = \sqrt{\frac{8000ε_0ε_rRT}{\sinh(zF\Psi_0/2RT)}} \times \sqrt{J}$$

(1)

where \(\Psi_0\) is the surface potential, \(R, T, F, z\) have their usual meanings (SI unit system). \(ε_0\) and \(ε_r\) are the permittivity and the dielectric constant, respectively. For small surface potentials, Eq. 1 can be linearized:

$$\sigma = zF\sqrt{2000ε_0ε_rRT} \times \Psi_0 \times \sqrt{J}$$

(2)

According to the Boltzmann distribution of the permeating ions, the local ion concentration at the membrane surface \(c_0\) is a function of the surface potential and the bulk ion concentration \(c_0\):

$$c_0 = c_0 \times \exp(-zF\Psi_0/RT)$$

(3)

As shown below, the “leak” fluxes appear as electroneutral transport (not depending on the transmembrane potential). In addition, as can be seen later, under the experimental conditions the “leak” fluxes show no evidence of saturation as concentration is increased. Therefore, assuming that the unidirectional flux of an ion species \(J\) is proportional only to the local ion concentration near the membrane surface,

$$J = k \times c_0$$

(4)

where \(k\) is the rate constant of ion translocation. One gets a dependence of the ion flux on the ionic strength of the medium (because the flux is usually
related to \( c_m \), the rate constant determined in experiments \( k^{\text{exp}} = \frac{J}{c_m} \):

\[
k^{\text{exp}} = k \times \exp \left( \frac{-\sigma^2}{2000\epsilon_0 RT} \right) \quad \text{with} \quad P = \frac{A}{V}
\]  

(5)

\( P, V, \) and \( A \) represent the membrane permeability due to the "leak" transport, the volume, and the surface area of the erythrocyte, respectively. Assuming a dielectric constant of water \( (\epsilon = 80) \) and taking into account \( T = 310 \) K, Eq. 5 will result in

\[
\log(k^{\text{exp}}) = 7.19\sigma / \sqrt{I} + \log(k)
\]  

(6)

where the units for \( I \) and \( \sigma \) are M and C/m², respectively.

### Numerical calculations and statistical treatment

The result of each flux experiment was calculated from the average of triplicate samples. Usually, each value presented as the final result represents the data \((\pm \text{SE})\) of at least three separate experiments carried out on the blood of different donors.

The fitting of Eqs. 6 and 19 to the experimental results were carried out using a nonlinear least-squares algorithm according to Marquardt-Levenberg, implemented with the program SigmaPlot v.2.01 (Jandel Corporation). For regression analysis in the Dixon plots, standard "Y on X" regression as well as geometric mean regression (Ricker, 1973) was used. The geometric mean regression takes into account errors in the dependent (1/flux) as well as in the independent (proton concentration) variable.

### RESULTS AND DISCUSSION

#### Electroneutrality of "leak" \( K^+ \) and \( Na^+ \) transport

To clarify the principal mechanism of the "leak" transport of monovalent cations across the erythrocyte membrane, the dependence of this transport on the transmembrane potential of the cell membrane must be taken into consideration. Previously it was shown by Bernhardt et al. (1991) that the transmembrane potential has no influence on the "leak" \( K^+ \) transport across the red blood cell membrane. Because this statement is of fundamental importance, additional investigations of the "leak" \( K^+ \) influx were carried out. The results are summarized in Table 1. Although after NaCl replacement by Na-tartrate or Na-glutonate the transmembrane potential changes significantly (comparable with the replacement of NaCl by sucrose), there is no alteration of the "leak" \( K^+ \) influx under such conditions. To show that a decrease in the Na⁺ concentration is not of importance for the large enhancement of the "leak" \( K^+ \) influx, NaCl was replaced by NMDG-Cl. In this case, only a slight increase in the flux was observed. The only parameter that seems to influence the flux substantially is the ionic strength of the extracellular solution (see below for detailed discussion). The finding that the transmembrane potential does not have an effect on the "leak" \( K^+ \) transport can be verified by comparing Ussing ratios for "leak" \( K^+ \) fluxes measured at different extracellular NaCl concentrations (sucrose replacement, constant osmolarity) and, therefore, at different transmembrane potentials, as shown in Fig. 1 and Table 2. The Ussing ratios obtained \((\approx 1.0)\) at different transmembrane potentials do not coincide with values that are expected for an electrogenic transport mechanism, but are in agreement with the assumption of a nonelectrogenic transport. For these calculations, the transmembrane potential of human erythrocytes was taken as the Nernst potential of the Cl⁻ distribution (with an intracellular Cl⁻ concentration of 110 mM). In addition, as one can estimate from Fig. 1, the Ussing ratios for the "leak" \( Na^+ \) fluxes \((k_{\text{eff}}, k_{\text{inf}})\) are \(\approx 1.0\).

#### "Leak" \( K^+ \) and \( Na^+ \) transport mediated by a \( K^+ \) (\( Na^+ \))/\( H^+ \) exchanger

To explain the electoneutrality of the "leak" \( K^+ \) transport, one must propose that for each cation transported another cation (or proton) must be exchanged, or an anion must be transported in the same direction. A simple \( K^+ /K^+ \) exchange mechanism can be excluded, because the unidirectional "leak" \( K^+ \) efflux \( \approx 20 \) times greater than the influx under all ionic strength conditions measured (Denner et al., 1993; Bernhardt, 1994). However, in HIS solutions one could assume an exchange of intracellular \( (K^+ + Na^+) \) against extracellular \( (K^+ + Na^+) \), because the "leak" cation \( (K^+ + Na^+) \) efflux \((2.92 \pm 0.41 \text{ mmol/(lcell h)})\) is in the same range as the influx \((1.64 \pm 0.20 \text{ mmol/(lcell h)})\) (Denner et al., 1993). On the other hand, under LIS conditions, a net "leak" cation efflux has been obtained (see also Donlon and Rothstein, 1969), i.e., the \((K^+ + Na^+) \) efflux \((20.02 \pm 1.64 \text{ mmol/})\)

### Table 1: Effect of NaCl replacement in physiological ionic strength (HIS) solution by different substances (constant osmolarity) on the "leak" \( K^+ \) influx in human red blood cells.

<table>
<thead>
<tr>
<th>Substance in the external solution</th>
<th>Ion concentration</th>
<th>Ionic strength</th>
<th>Calculated ( \Delta \Psi_m )</th>
<th>( K^+ ) influx</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Na⁺ (mM)</td>
<td>Cl⁻ (mM)</td>
<td>Na⁺ (mM)</td>
<td>Cl⁻ (mM)</td>
</tr>
<tr>
<td>NaCl (HIS)</td>
<td>145</td>
<td>152.5</td>
<td>165</td>
<td>-9</td>
</tr>
<tr>
<td>Sucrose (LIS)</td>
<td>0</td>
<td>7.5</td>
<td>20</td>
<td>+70</td>
</tr>
<tr>
<td>NMDG-Cl</td>
<td>0</td>
<td>152.5</td>
<td>165</td>
<td>-9</td>
</tr>
<tr>
<td>Na-tartrate</td>
<td>214</td>
<td>7.5</td>
<td>331</td>
<td>+70</td>
</tr>
<tr>
<td>Na-tartrate + sucrose</td>
<td>110</td>
<td>7.5</td>
<td>165</td>
<td>+70</td>
</tr>
<tr>
<td>Na-glutonate</td>
<td>145</td>
<td>7.5</td>
<td>165</td>
<td>+70</td>
</tr>
</tbody>
</table>

NaCl (145 mM) was replaced by 250 mM sucrose, 145 mM NMDG-Cl, 145 mM Na-glutonate, 107 mM Na-tartrate, or 55 mM Na-tartrate plus 120 mM sucrose. Flux solutions contained, additionally, 7.5 mM KCl and 5.8 mM NaH₂PO₄/Na₂HPO₄, pH 7.4 (considered in the given Cl⁻ concentration and the ionic strength), and 10 mM glucose. The transmembrane potential \( (\Delta \Psi_m) \) was calculated according to the Nernst potential for Cl⁻ distribution \((A)\) and according to Glaser (1979) (B).
of inhibitors constant 5.7 mM FIGURE 1 TABLE 2 leads erythrocytes, to according K+/Cl− concentration is lower the membrane surface. These additionally, in LIS media the more negative external surface potential (in comparison to HIS media) of the erythrocytes, leads to a decreased anion concentration near the membrane surface (as can be seen below). This would further lower the transport rate of a cation/anion cotransport under LIS conditions (which, e.g., can be seen for the loss of the Na+/K+/2Cl− cotransport activity in LIS solutions; data not shown). However, one could imagine a cation/anion cotransport with a very high affinity toward the anion. Assuming such a high-affinity binding site for the anions (and a low-affinity binding site for the cations; see below) at both sides of the membrane would result in an identical cation efflux and influx (proportional to the product of the extracellular and intracellular cation concentrations), which is not the case under LIS conditions. If, however, only the external anion binding site is saturated, the cation influx is expected to be larger than the efflux. For the opposite situation (high intracellular affinity for the anion), the cation efflux should be larger than the influx, which is in fact the case under LIS conditions. However, this would lead to a decrease in both the cation influx and efflux when the extracellular anion concentration is reduced. Therefore, we do not consider a cation/anion cotransport to be the basis of the electroneutral “leak” K+ and Na+ fluxes. The only reasonable explanation for an electroneutral balance of the net (K+ + Na+) efflux is a proton influx. This can be realized simply by a K+(Na+)/H+ exchanger.

As an extension of the previously published carrier model explaining the “leak” transport for K+ and Na+ (Denner et al., 1993), the influence of the ionic strength as well as the competition of Na+ and K+ has been investigated in more detail. If one assumes that the reduction of the Na+ concentration is solely responsible for the increase in the “leak” K+ influx under LIS conditions (NaCl replacement by sucrose), this effect should also be observed after replacement of NaCl by any other substance that does not contain Na+. (lcell(h)) is significantly higher than the (K+ + Na+) influx (1.82 ± 0.22 mmol/(lcell(h))) (Denner et al., 1993).

Because the “leak” K+ influx increases at reduced ionic strength but does not necessarily decrease when the Cl− concentration is dramatically reduced (Table 1, Fig. 1), an electroneutral K+/Cl− cotransport pathway can be ruled out. Additionally, in LIS media the more negative external surface potential (in comparison to HIS media) of the erythrocytes, leads to a decreased anion concentration near the membrane surface (as can be seen below). This would further lower the transport rate of a cation/anion cotransport under LIS conditions (which, e.g., can be seen for the loss of the Na+/K+/2Cl− cotransport activity in LIS solutions; data not shown). However, one could imagine a cation/anion cotransport with a very high affinity toward the anion. Assuming such a high-affinity binding site for the anions (and a low-affinity binding site for the cations; see below) at both sides of the membrane would result in an identical cation efflux and influx (proportional to the product of the extracellular and intracellular cation concentrations), which is not the case under LIS conditions. If, however, only the external anion binding site is saturated, the cation influx is expected to be larger than the efflux. For the opposite situation (high intracellular affinity for the anion), the cation efflux should be larger than the influx, which is in fact the case under LIS conditions. However, this would lead to a decrease in both the cation influx and efflux when the extracellular anion concentration is reduced. Therefore, we do not consider a cation/anion cotransport to be the basis of the electroneutral “leak” K+ and Na+ fluxes. The only reasonable explanation for an electroneutral balance of the net (K+ + Na+) efflux is a proton influx. This can be realized simply by a K+(Na+)/H+ exchanger.

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but is suitable for maintaining osmolarity (and ionic strength). To elucidate such a possible K⁺/Na⁺ competition and to underline the effect of the ionic strength on the “leak” K⁺ transport, we used NMDG⁺ for a gradual replacement of Na⁺. Under these conditions, the ionic strength of the extracellular solution remains constant. In parallel, the ionic strength has been decreased by a gradual replacement of NMDG-Cl by sucrose, comparable to earlier studies in which NaCl was replaced by sucrose. The results presented in Fig. 2 demonstrate that the increase in the “leak” flux in LIS solution is only negligibly an effect of competition of Na⁺ and K⁺ at the ion concentrations investigated, but is predominantly due to the reduction of the physical parameter ionic strength.

The relatively small competition of K⁺ and Na⁺ can be demonstrated from a Dixon plot of experimental results presented in Fig. 3, showing a large inhibition constant of Na⁺ for the “leak” K⁺ influx (for discussion of the suitability of the Dixon plot under such conditions, see below). Defining the point of intersection of curves measured at different K⁺ concentrations in the extracellular medium as (−K⁺Na⁺, 1/VNa⁺), one estimates from Fig. 3, K⁺Na⁺ ≈ 600 mM and VNa⁺ ≈ 1.5 mmol/(l cell h).

Taking into account that the K⁺ influx is slightly inhibited by extracellular Na⁺ (as well as the electroneutrality of the “leak” Na⁺ fluxes), it seems plausible that the “leak” Na⁺ transport is also mediated by a K⁺(Na⁺)/H⁺ exchanger. Such a K⁺(Na⁺)/H⁺ antiporter has been reported for mitochondrial membranes (Ježek et al., 1990). A K⁺/H⁺ exchanger is present in *Amphiiuma* and fish red blood cells (Adorante and Cala, 1987; Fievet et al., 1993), but the direct involvement of Na⁺ in this transport pathway has not yet been clarified.

To demonstrate the influence of the H⁺ concentration on the proposed K⁺(Na⁺)/H⁺ exchanger, we designed experiments in which cis protons are treated as an inhibitor of the “leak” cation flux (for the influence of *trans* protons, see the discussion of transport models). In enzyme kinetics, an inhibitor is usually characterized by using a Dixon plot. In membrane transport studies, especially when protons are involved, the situation is more complicated, because one must take into account the substrate and inhibitor concentrations on both sides of the membrane, i.e., including the situation on the *trans* side. However, in analogy with enzyme kinetics, appropriate plots (like a Dixon plot) can be used to analyze the experimental transport data (Ohki and Spangler, 1991). The inverse of the flux measured in solutions of different ionic strengths is plotted against the *cis* proton concentration (Figs. 4 and 5). This procedure was carried out for fluxes measured in two different buffer systems with constant bulk K⁺ concentration in the flux solutions (7.5 mM; see Materials and Methods).

As can be seen from Figs. 4 and 5, extracellular protons act as an inhibitor of the “leak” K⁺ influx under all ionic strength conditions. However, one obtains different slopes of the regression lines at different ionic strengths. Because different substrate concentrations lead to different slopes in a Dixon plot, we conclude that the main influence of the ionic strength is to alter the substrate concentration (K⁺) near the external membrane surface. Further evidence is presented below.

Defining the intercept of the point of intersection of the curves measured at different ionic strengths (i.e., different K⁺
as $1/V_H^\text{max}$, one can roughly estimate these parameters from Fig. 4 as well as from Fig. 5. The values of $K_H^\text{app}$ and $V_H^\text{max}$ are $<10^{-8}$ M and $>30$ mmol/(lcells h), respectively. For $V_H^\text{max}$, only a lower limit can be given, because this parameter should only be read at saturating K⁺ concentrations, which most probably are not present under our conditions.

Of course, one could imagine an alternative approach to investigating the proposed involvement of the H⁺ transport. However, a direct measurement of changes in pH of the external solution in parallel to the K⁺ flux experiments was not applicable because of the fast H⁺ (OH⁻) transport via the band 3 protein, even under conditions in which the anion exchange is inhibited. The calculated H⁺ efflux corresponding to the measured extracellular pH change in 4,4'-disothio-cyanostilben-2,2'-disulfonic acid (DIDS)-treated red blood cells is still larger than the “leak” K⁺ influx under LIS conditions (data not shown). This result indicates that there is a H⁺ translocation across the membrane, not only via the band 3 protein and the described K⁺(Na⁺)/H⁺ exchanger, but also via other transport pathways (at least in HCO₃⁻-containing media).

### Influence of the surface potential

The importance of surface charge effects on ion transport phenomena has already been reported (Neumcke, 1970; McLaughlin et al., 1970; Theuvenet and Borst-Pauwels, 1976; McLaughlin, 1977; Dani, 1986; Jordan, 1987; Riddell and Arumugam, 1988; Green and Andersen, 1991). To demonstrate the quantitative effect of the ion concentration near the external cell surface (i.e., the membrane surface potential) on the “leak” K⁺ and Na⁺ influxes, the rate constants of these fluxes were plotted (semilogarithmic) against the inverse of the square root of the ionic strength of the extracellular solution. According to the linearized version of the Gouy-Chapman theory (cf. Theoretical Background, above), the assumption that the surface potential is important for the increase in the “leak” fluxes should result in straight lines for such plots (cf. Eq. 6). Because of the assumed K⁺(Na⁺)/H⁺ exchange process (see above, and below, Eq. 13), the “leak” K⁺ and Na⁺ effluxes, like the influxes, depend on the ion concentration near the external cell surface. As one can see from the inset of Fig. 1 for all four unidirectional “leak” fluxes (K⁺ and Na⁺ influx, K⁺ and Na⁺ efflux), this assumption does hold. The linear correlation coefficients for the four plots are $\geq0.98$.

From the slope of the lines and their intersections shown in the inset of Fig. 1, it is possible to calculate the surface charge density ($\sigma$) and the rate constant of the flux, respectively. The rate constants have been converted into membrane permeabilities due to the “leak” transport of the corresponding ions, assuming a volume/surface ratio of $1.4 \times 10^{-6}$ m. Table 3 lists the values obtained for the “leak” K⁺ and Na⁺ influxes as well as effluxes. The apparent permeabilities are on the same order of magnitude as values obtained from the classical concept of electrodiffu-
sion (Lew and Beauge, 1979). The values for $\sigma$ represent the average surface charge density in the proximity of the transport site(s) and not necessarily of the whole cell surface. Values of effective (local) surface charge densities of cloned K$^+$ channels in *Xenopus* oocytes in the range of $-0.017$ C/m$^2$ and $-0.050$ C/m$^2$ were obtained (Elinder et al., 1996). However, the obtained values for $\sigma$ in the present paper are also close to values of the surface charge density of human erythrocytes reported in the literature ($-0.019$ C/m$^2$; see, e.g., Donath et al., 1996).

Interestingly, the calculation of the surface charge density provides slightly different values when the data for the K$^+$ or Na$^+$ fluxes are used (Table 3). This could be due to differences in the binding constants for K$^+$ and Na$^+$ at the transport site(s) of the carrier, or it could reflect differences in the ionic size of K$^+$ (Rb$^+$) and Na$^+$. The first explanation is based on the fact that the ratio of the unidirectional fluxes (Na$^+$/K$^+$) is proportional to the ratio of the binding constants of the corresponding ions (under nonsaturating conditions; as assumed for the transporter, cf. Eq. 4; this is reasonable, in view of the high apparent inhibition constant for Na$^+$, cf. Fig. 3). For the second possibility, it would be expected that under LIS conditions, the differences in K$^+$ and Na$^+$ fluxes are less marked than in HIS media. In LIS media, the Debye-length increases would abolish the difference between K$^+$ and Na$^+$ ion size. In fact, a comparison of the rate constants for K$^+$ and Na$^+$ fluxes as well as fluxes shows that in LIS media, the ratio of K$^+$ to Na$^+$ fluxes is $\approx 1$, whereas at physiological ionic strength the ratio is $\approx 4$. The dependence of the ratio of the rate constants (Na$^+$/K$^+$ influx, Na$^+$/K$^+$ efflux) on the ionic strength of the extracellular solution ($I$) can be described by a single linear regression line $k_{\text{Kflux}}/k_{\text{Naflux}} = 0.11 \times I^{-0.5} [M^{-0.5}] + 0.22$ (linear correlation coefficient $r^2 = 0.95$).

Of course, there are some other possible explanations of the difference in calculated surface charge density affecting the “leak” K$^+$ and Na$^+$ fluxes: 1) The transport site for the cations may be negatively charged, or its immediate environment contributes to the calculated value of the surface charge density. Such a discrete charge effect would result in a dependency of the ion binding on both the ionic strength and the distance of the ion to the charge near the transport site (determined by the ion size) (Nelson and McQuarrie, 1975). In agreement with this view is the fact that in the hydrated form, the ion size for Na$^+$ is smaller than that of K$^+$. 2) It cannot be excluded that at HIS, all known specific transport pathways involving Na$^+$ are not completely inhibited (see also Introduction). For example, Na$^+/H_2PO_4^-$ (NaHPO$_4^-$) cotransport (Shoemaker et al., 1988) could play a role in HIS but not in LIS media (because of the reduced concentration of Na$^+$).

To illustrate the effect of the ionic strength of the extracellular solution, we calculated the K$^+$ and Na$^+$ concentration near the transport site, using Eqs. 2 and 3 as well as the estimated surface charge densities ($-0.024$ C/m$^2$ for K$^+$ and $-0.014$ C/m$^2$ for Na$^+$). As one can see from Fig. 6, under LIS conditions (ionic strength $\approx 0.017$ M) there is an eightfold increase in the K$^+$ concentration near the transport site at the membrane surface compared to the K$^+$ concentration in HIS solution. A comparable increase in the “leak” K$^+$ influx (eightfold) can be obtained in a physiological ionic strength solution when the bulk K$^+$ concentration is enhanced from 7.5 mM to 72.5 mM (increase calculated from values taken from Fig. 3 at zero extracellular Na$^+$ concentration).

Further evidence for the influence of the surface charge on the “leak” K$^+$ influx is presented in Fig. 7. The intercalation of a negatively or positively charged amphiphilic substance (SDS and DDTMA, both with an alkyl chain length of 12 carbon atoms) into the erythrocyte membrane does not result in a remarkable change in the influx in HIS solution (in the concentration range studied, 0–100 $\mu$M). In contrast, under LIS conditions, the insertion of the negatively charged amphiphilic substance leads to a significant increase in the “leak” K$^+$ influx, whereas the positively charged substance causes a decrease in the flux. The relative increase in the “leak” K$^+$ influx at higher DDTMA concentrations is not surprising, because it is known that the interaction of amphiphilic substances with the erythrocyte

---

TABLE 3 Surface charge density ($\sigma$) and membrane permeability due to the “leak” K$^+$ and Na$^+$ transport (P) obtained from fits of Eq. 6 to the K$^+$ and Na$^+$ influxes as well as effluxes (cf. inset of Fig. 1)

<table>
<thead>
<tr>
<th></th>
<th>K$^+$ influx</th>
<th>K$^+$ efflux</th>
<th>Na$^+$ influx</th>
<th>Na$^+$ efflux</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\sigma$ (C/m$^2$)</td>
<td>$-0.024$</td>
<td>$-0.025$</td>
<td>$-0.014$</td>
<td>$-0.016$</td>
</tr>
<tr>
<td>$P \times 10^{12}$ (ms$^{-1}$)</td>
<td>1.5</td>
<td>1.7</td>
<td>4.4</td>
<td>4.8</td>
</tr>
</tbody>
</table>

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![FIGURE 6 Ratio of the cation concentration near the membrane surface at the ionic strength (given on the *abscissa*) to the cation concentration near the membrane surface at physiological ionic strength (160 mM). The calculations have been performed using Eqs. 2 and 3, for the K$^+$ concentration with $\sigma = -0.024$ C/m$^2$ (*upper curve*) and for the Na$^+$ concentration with $\sigma = -0.014$ C/m$^2$ (*lower curve*). For values of $\sigma$ cf. Table 3. The insets shows the dependence of the K$^+$ (*solid line, left axis*) and Na$^+$ (*dotted line, right axis*) concentration near the membrane surface on the extracellular bulk Na$^+$ concentration (i.e., at different ionic strength). The ionic strength of the buffer substance was assumed to be 0.01 M, and a bulk KCl concentration of 7.5 mM was taken into consideration.](http://example.com/fig6)
Comparison of competitive and noncompetitive K\textsuperscript+ (Na\textsuperscript+)/H\textsuperscript+ transport

To investigate the mechanism of the K\textsuperscript+ (Na\textsuperscript+)/H\textsuperscript+ exchange in more detail, the experimental results are analyzed in terms of two different kinetic transport models shown in Fig. 8. For the derivation of the equations according to the transport models, we made the following assumptions: 1) the binding of the ions to the transporter is close to equilibrium, i.e., the rate constants of association and dissociation of the ions are much higher than the rate constants of translocation (k); 2) the rate constants of translocation are independent of the direction of motion (k\textsubscript{apo} = k\textsubscript{aop}); 3) under steady-state conditions, the distribution of the carrier at the two membrane interfaces is time independent; 4) the total carrier concentration (C\textsubscript{tot}) remains constant.

The binding constant

\[ K_X = \frac{[CX]}{[C] \times [X]} \]  

where [X] is the concentration of a substrate (ion), [C] is the concentration of the free carrier, and [CX] is the concentration of the bound substrate (ion), can be used to transform the ion concentrations into “normalized” concentrations:

\[ c_X = K_X \times [X] = [CX]/[C]. \]

This was done to simplify the equations of the corresponding models. In the following equations, subscripts o and i indicate the external and internal concentrations, respectively. For convenience, it was also assumed that the rate constants of translocation for the transition between the membrane interfaces are all the same (independent of the ion species), and therefore are equal to k. This assumption was used to reduce the number of terms in the equations describing the fluxes. However, it does not qualitatively change the dependence of the fluxes on the ion concentrations. Such a procedure seems plausible because it maintains the relationship between the flux and the external ion concentrations, and because the phenomenological parameters obtained from the Dixon plot (k\textsuperscript{app}\textsubscript{H}, k\textsuperscript{app}\textsubscript{Na}, V\textsubscript{H\textsuperscript{max}}, V\textsubscript{Na\textsuperscript{max}}) cannot be mapped to the parameters describing the model and, therefore, differences in translocation constants are confounded with the apparent binding constants.

Antiport model with competitive binding of K\textsuperscript+, Na\textsuperscript+, and H\textsuperscript+

According to the model presented in Fig. 8 A, one obtains

\[ f_{K}^{\text{flux}} = (C_{\text{tot}}c_K)(c_{H} + c_{K^+} + c_{Na^+})/D_2 \]  

\[ f_{K}^{\text{flux}} = (C_{\text{tot}}c_K)(c_{H} + c_{K^+} + c_{Na^+})/D_2 \]
with

\[ D_2 = \left[ (1 + 2(c_{H} + c_{K} + c_{Na})) \right] \times c_{H} \]
\[ + \left[ (1 + 2(c_{H} + c_{K} + c_{Na})) \times (c_{K} + c_{Na}) \right] \times c_{H} \]
\[ + c_{H} + c_{K} + c_{Na} \]

(11)

From Eqs. 9 and 10 it follows that both the K⁺ influx and the K⁺ efflux increase in the same proportion when the ionic strength of the extracellular solution is reduced (the reduction of the ionic strength leads to an increase in the cation concentration near the membrane surface (cf. Eqs. 2 and 3). Equation 9 can be transformed to give the inverse of the K⁺ influx, consisting of one term determining the slope and of another term determining the intercept, in a Dixon plot (1/J_{K}^{influx} versus c_{H}):

\[ \frac{1}{J_{K}^{influx}} = \frac{(2 + f_1)}{C_{tot}k_{C}c_{Ko}} \times c_{H} + \frac{1 + ((2 + f_1) \times (c_{K} + c_{Na}))}{C_{tot}k_{C}c_{Ko}} \]

(12)

with

\[ f_1 = \frac{1}{c_{H} + c_{K} + c_{Na}} \]

As can be seen, the slope of the curve in a Dixon plot according to Eq. 11 would not be affected by changes in the extracellular Na⁺ concentration or by changes in the ionic strength of the extracellular solution. Because the ionic strength will affect c_{H} and c_{K} identically, it will be canceled out in Eq. 11. This is not in agreement with the experimental results (Figs. 4 and 5).

**Antiport model with noncompetitive binding of (K⁺ or Na⁺) and H⁺**

In contrast to the model discussed above, the model presented in Fig. 8 B does not involve competition in binding of K⁺ or Na⁺ and H⁺. The carrier is capable of binding both K⁺ or Na⁺ and H⁺, but in this state only one ion species can be transported (competition for being transported). For this model the following equations can be derived:

\[ J_{K}^{influx} = \left( \frac{C_{tot}k_{C}c_{Ko}(c_{H} + c_{K} + c_{Na})}{D_3} \right) \]

(13)

\[ J_{K}^{efflux} = \left( \frac{C_{tot}k_{C}c_{Ko}(c_{H} + c_{K} + c_{Na})}{D_3} \right) \]

(14)

with

\[ D_3 = \left[ (2c_{H} + 2c_{K} + 2c_{Na} + 1 + c_{H}c_{K} + c_{H}c_{Na}) \right] \times c_{H} \]
\[ + (c_{K} + c_{Na}) \times (c_{H} + c_{K} + c_{Na}) \times c_{H} \]
\[ + (c_{K} + c_{Na}) \times (2c_{H} + 2c_{K} + 2c_{Na}) \]
\[ + 1 + c_{H}c_{K} + c_{H}c_{Na} + c_{H} + c_{K} + c_{Na} \]

Similar to the model with only competitive binding (Fig. 8 A), it also follows from Eqs. 12 and 13 that both the K⁺ influx and the K⁺ efflux show the same relative increase when the ionic strength of the extracellular solution is reduced. As in the model discussed above, one obtains a linear dependence of the inverse of the K⁺ influx as a function of the external H⁺ concentration:

\[ \frac{1}{J_{K}^{influx}} = \frac{(c_{K} + c_{Na} + 2 + f_2)}{C_{tot}k_{C}c_{Ko}} \times c_{H} \]
\[ + \frac{1 + (c_{K} + c_{Na})(2 + f_2)}{C_{tot}k_{C}c_{Ko}} \]

with

\[ f_2 = \frac{1 + c_{H}(c_{K} + c_{Na})}{c_{H} + c_{K} + c_{Na}} \]

In contrast to the model with only competitive binding, the slope of the curve in a Dixon plot (external protons as inhibitor) is a function that can be split into two terms, one dependent on and one independent of the external ion concentrations (K⁺ and Na⁺). Therefore, there will be an influence of the ionic strength of the extracellular solution as well as of the extracellular sodium concentration on the slope of the curve, which is in agreement with the experimental results (Figs. 4 and 5).

In addition, one can relate the parameters \( K_{H}^{app} \) and \( V_{H}^{max} \) obtained from Fig. 4 and Fig. 5 to the kinetic constants of the noncompetitive transport model (Fig. 8 B). From Eq. 14 it follows that

\[ K_{H}^{app} = \frac{1}{K_{H}} \left( 1 + \frac{1 + c_{Na}(2 + f_2)}{c_{Na} + 2 + f_2} \right) \]

(16)

and

\[ V_{H}^{max} = \frac{C_{tot}k_{C}}{2 + f_2} \]

(17)

Both parameters depend on the intracellular conditions \( (c_{Na}, c_{K}, c_{H} \text{ combined in } f_2) \), and \( K_{H}^{app} \) depends on the Na⁺ binding. Therefore, the estimated values of \( K_{H}^{app} \) and \( V_{H}^{max} \) do not reflect “true” values for the proton binding constant and the saturated K⁺ flux, respectively.

One problem that must be discussed is the internal H⁺ concentration (see Eq. 14). As long as the internal pH is constant, there is no deviation from the linearity with respect to the external proton concentration in Eq. 14. Usually the intracellular pH is set by the extracellular conditions. The normalized intracellular H⁺ concentration is proportional to \( c_{H}^{\alpha} \) with \( 0 \leq \alpha \leq 1 \), depending on the transmembrane potential and the intracellular (hemoglobin) and extracellular buffer capacities. This could result in a deviation from linearity (Eq. 14) and might be expected to give a less satisfactory linear fit to this equation, which was not in fact found (Figs. 4 and 5). It seems reasonable to assume, therefore, that for each line of the Dixon plots (Figs. 4 and 5), there is no obvious change in slope. This could be due to the fact that the factor \( f_2 \) in Eq. 14 is a saturating function of
the internal proton as well as K\textsuperscript{+} and Na\textsuperscript{+} concentrations (cf. Fig. 9).

To investigate the influence of the trans proton concentration on the “leak” K\textsuperscript{+} influx, experiments at different intracellular pH (pH\textsubscript{i}) but at the same extracellular pH were carried out. To inhibit the anion exchanger (band 3), dipyr-ridamole (50 \textmu M) was used. DIDS does not seem to be applicable, because it is known that it interacts with the K\textsuperscript{+}/H\textsuperscript{+} exchanger present in the Amphiuma red blood cell membrane (Maldonado and Cala, 1994). At the beginning of the flux period, the pH\textsubscript{i} of erythrocytes adjusted with HCl and NaOH was 6.5 and 7.6, respectively (pH\textsubscript{i} of control cells was 7.0). The pH\textsubscript{i} at the beginning of the flux period was 7.4 ± 0.1 at all pH\textsubscript{i} investigated. However, under all pH\textsubscript{i} conditions, the “leak” K\textsuperscript{+} influx does not change significantly (flux values in the range from 0.15 to 0.17 mmol/leuc(hi); three independent experiments). This result is in agreement with the assumption that the intracellular pH does not have a significant influence on the “leak” K\textsuperscript{+} influx and, therefore, on the slopes of the lines in the Dixon plots (Figs. 4 and 5). In addition, in the presence of DIDS, the “leak” K\textsuperscript{+} efflux does not show a significant change upon alteration of the extracellular (trans) pH in the range 6.5–8.5, but depends on the intracellular (cis) pH (Chipperfield and Shennan, 1986).

One could expect that a trans proton stimulation of the flux should be found for the K\textsuperscript{+}(Na\textsuperscript{+})/H\textsuperscript{+} exchanger. This would be true for the transport model with competitive binding of the substrates (Fig. 8 A). In contrast, for the model with noncompetitive binding of K\textsuperscript{+}(Na\textsuperscript{+}) and H\textsuperscript{+} (Fig. 8 B), this does not hold under all conditions. Analyzing the dependence of the K\textsuperscript{+} influx on the intracellular H\textsuperscript{+} concentration (Eq. 12), one gets a trans stimulation (presented in Fig. 10) if \(b \times c > a \times d\), with

\[
b \times c = (2c_{H_i} + 2c_{K_i} + 2c_{Na} + 1 + c_{H_i}c_{K_i} + c_{H_i}c_{Na})
\]

\[
a \times d = (2c_{H_i} + 2c_{K_i} + 2c_{Na} + 1 + c_{H_i}c_{K_i} + c_{H_i}c_{Na})
\]

\[
\times (c_{K_i} + c_{Na}) + (c_{H_i} + c_{K_i} + c_{Na})
\]

\[
\times (c_{K_i} + c_{Na})^2
\]

Equations 17 and 18 were obtained by rearranging the nominator (\(D_3\)) in Eqs. 12 and 13. The rearrangement can be simplified by taking efflux as influx as well as changing the subscripts i and 0 for o and i, respectively. As one can see, trans proton stimulation of the influx will be present only if \(c_{K_i} + c_{Na} < 1\). Taking into account that \(c_{K_i}, c_{K_o}, c_{Na}, c_{Na_i}\), and \(c_{Na_o}\) are normalized concentrations, it follows that they are <1 if the chemical concentration of the corresponding ion is smaller than the dissociation constant (inverse of the binding constant). If \(c_{K_i} + c_{Na} > 1\), one gets a trans inhibition. This result is not surprising, because at high alkali metal ion concentration (trans), the transporter is trapped in a configuration in which no transport occurs (e.g., KC\textsubscript{i}H and NaC\textsubscript{i}H for influx; cf. Fig. 8 B). Nevertheless, it is

**FIGURE 9** Dependence of the term \(f_i\) in Eq. 14 on the normalized intracellular H\textsuperscript{+} concentration (C\textsubscript{H\textsubscript{i}}) at different normalized intracellular (K\textsuperscript{+} + Na\textsuperscript{+}) concentrations (\(c_{K_i} + c_{Na}\)). (A) C\textsubscript{H\textsubscript{i}} = 2. (B) C\textsubscript{H\textsubscript{i}} = 0.5. (C) C\textsubscript{H\textsubscript{i}} = 0.5. 

**FIGURE 10** Dependence of the unidirectional K\textsuperscript{+} flux (influx or efflux) on the cis and trans proton concentration (presented schematically). The parameters m, n, a, b, c, and d of the equations represent sums of terms of Eqs. 9 and 10 or Eqs. 12 and 13 (corresponding to the competitive and noncompetitive K\textsuperscript{+}(Na\textsuperscript{+})/H\textsuperscript{+} transport model, respectively). One gets trans stimulation of K\textsuperscript{+} transport if \(b \times c > a \times d\), trans inhibition if \(b \times c < a \times d\). The flux remains constant if \(b \times c = a \times d\). For the noncompetitive transport model, see Eqs. 17 and 18.
most likely that the condition for the trans proton stimulation is fulfilled under the experimental conditions, because the transporter is far from saturation with regard to K\(^+\) and Na\(^+\) (see above). However, on the basis of the experimental results (low \(K_{H}^{\text{app}}\), no significant change in “leak” K\(^+\) influx at different pH\(_{i}\)), it is reasonable to assume that the influence of the intracellular proton concentration on the “leak” K\(^+\) influx has reached saturation (cf. Fig. 10). This is also in agreement with the fact that there is no obvious change in the slope of the lines in the Dixon plots (as already discussed for the factor \(f_2\)). Interestingly, because cis proton inhibition is predicted under all conditions, alteration of both intracellular and extracellular proton concentrations shows a dominant influence of the cis dependence at high proton concentration (compared to the proton dissociation constant; cf. equations in Fig. 10).

In addition to the presentation of the experimental results (Figs. 4 and 5), a direct fit of the measured “leak” K\(^+\) influx dependence on the K\(^+\) and H\(^+\) concentrations near the outer cell surface was carried out. For the calculation of the K\(^+\), Na\(^+\), and H\(^+\) concentrations near the outer surface, an average surface charge density of \(-0.019 \text{ C/m}^2\) was used (estimated value according to the inset of Fig. 1; see also Table 3) with Eq. 19 (derived from Eq. 12):

\[
J_{\text{K}}^{\text{influx}} = \frac{C_{\text{tot}} k_4 [K^+]_o K_{K}}{D_4}
\]

with

\[
D_4 = [H^+]_o K_{H} (2 + f_2) + [K^+]_o K_{K} + [Na^+]_o K_{Na}
+ 1 + ([K^+]_o K_{K} + [Na^+]_o K_{Na}) (2 + f_2)\]

Assuming \(K_{Na} = K_{K} = 1/0.6 \text{ M}^{-1}\) (taken from Fig. 3) and \(f_2 = 1\), one obtains \(1/K_{H} = 8 \times 10^{-7} \text{ M}\) and \(C_{\text{tot}} k_4 = 180 \text{ mmol/(lcell h)}\) (Fig. 11). Differences in the values reflecting the proton binding \((K_{H}^{\text{app}}, 1/K_{H})\) and the maximum transport rate \((V_{\text{Na}}^{\text{max}}, C_{\text{tot}} k_4)\) obtained from the Dixon plots (Figs. 4 and 5) and the direct fit (Fig. 11) are plausible, taking into consideration Eqs. 15 and 16. Furthermore, for the Dixon plots, the bulk proton concentration, not the proton concentration near the membrane surface, was used. However, the proton binding constant and the maximum transport rate obtained from the Dixon plots and the direct fit are on the same order of magnitude. Although we do not know the exact values of the parameters \(K_{K}, K_{Na}\), and \(f_2\), it must be stated that reasonable values other than those used above do result in changes in the estimated parameters but do not qualitatively change the shape of the curve shown in Fig. 11. In addition, because the flux measurements cannot be carried out under unsaturated conditions, the estimated parameters represent only a rough estimate.

In the transport model presented in Fig. 8 B, a competitive inhibition (binding) between K\(^+\) and Na\(^+\) is assumed. Therefore, one obtains the following linear function of the inverse of the K\(^+\) influx for the external Na\(^+\) concentration:

\[
\frac{1}{J_{\text{K}}^{\text{influx}}} = \frac{c_{H}}{C_{\text{tot}} K_{K} k_4} \times c_{Na}
+ \frac{(c_{H} + c_{K})(2 + f_2) + c_{H} c_{K} + 1}{C_{\text{tot}} K_{K} k_4}
\]

which fits the experimental results shown in Fig. 3. Furthermore, from Eq. 20 one can conclude that the change in the slope of the curves measured at different \(c_{K}\) shown in Fig. 3 should be inversely proportional to the change in the external K\(^+\) concentration. In fact, the ratios of the extra-cellular K\(^+\) concentrations (7.5 mM/2.5 mM = 3 and 72.5 mM/7.5 mM = 9.7) coincide with the inverse ratios of the corresponding slopes of the three lines shown in Fig. 3 (3 \(\pm\) 1.6 and 14.1 \(\pm\) 4.1). Therefore, it seems reasonable to assume a competitive type of inhibition between Na\(^+\) and K\(^+\).

Again, the parameters \(K_{l}^{\text{app}}\) and \(V_{\text{Na}}^{\text{max}}\) are apparent parameters that are not directly related to the Na\(^+\) binding and the “true” \(C_{\text{tot}} k_4\) of the carrier. The relations can be seen in Eqs. 21 and 22:

\[
K_{l}^{\text{app}} = \frac{1 + c_{H}(2 + f_2)}{K_{Na}} \times c_{H} + 2 + f_2
\]

\[
V_{\text{Na}}^{\text{max}} = \frac{c_{\text{tot}} k_4}{2 + f_2 + c_{H}}
\]

The obtained \(V_{\text{Na}}^{\text{max}}\) is much smaller than \(V_{\text{H}}^{\text{max}}\) (see above). Indeed, a comparison of Eqs. 22 and 16 shows that \(V_{\text{Na}}^{\text{max}}\) estimated from Fig. 3 should be smaller than \(V_{\text{H}}^{\text{max}}\) estimated from Figs. 4 and 5. However, because of the great distance of the estimated point of intersection from the measured data points in Fig. 3, the error in \(V_{\text{Na}}^{\text{max}}\) is too large for a conclusive statement in this respect.

The result obtained for the proton binding is in good agreement with data reported in the literature for other monovalent cation/proton exchangers, e.g., for the plasma...
membrane Na\(^+\)/H\(^+\) exchanger \(K_{1/2}^{\text{H}} = 10^{-8}\) to \(10^{-7}\) M (Aronson, 1985). In contrast, the estimated apparent inhibition constant for Na\(^+\) is at least one order of magnitude larger than the value reported for the Na\(^+\)/H\(^+\) exchanger (\(K_{1/2}^{\text{Na}} = 10^{-3}\) to \(10^{-2}\) M). It is not clear to what extent this fact is based on differences between apparent and "true" parameters or reflects the characteristics of the Na\(^+\) binding of the described transporter. However, the relatively large value of \(K_{1/2}^{\text{Na}}\) (and, therefore, probably a large value of \(K_{1/2}^{\text{Na}}\)), together with a relatively low value of \(K_{1/2}^{\text{H}}\), could be the reason for the finding that the "leak" fluxes of monovalent cations at physiological ion concentrations do not show saturation (Bernhardt et al., 1991).

**CONCLUSION**

The principal conclusion of the present work is that the "leak" transport of K\(^+\) and Na\(^+\) across the human erythrocyte membrane can be described as an electroneutral exchange of cis cations against trans protons or cations (K\(^+\)(Na\(^+\))/H\(^+\) exchange). It is unlikely that the protons compete for the same binding site as K\(^+\) (noncompetitive binding). In contrast, Na\(^+\) and K\(^+\) probably compete for the same binding site.

This transport depends strongly on the local ion concentrations near the membrane surface, which is manifested by increased ion fluxes (influx and, because of the exchange process, efflux) upon the decrease in the ionic strength of the external solution. Furthermore, the transport system operates far from saturation under physiological conditions.

Considering the fact that sickle cells and xerocytes show increased but balanced "leak" K\(^+\) and Na\(^+\) fluxes not leading to a cation depletion of the cells after inhibition of the Na\(^+\) pump (Joiner et al., 1986), and, therefore, suggesting an electroneutral ion transport pathway for these pathological cells, the involvement of the K\(^+\)(Na\(^+\))/H\(^+\) exchanger seems conceivable. Further investigations of red blood cell with altered "leak" cation fluxes (sickle cells, xerocytes, malaria-infected red blood cells, erythrocytes from patients with specific types of hypertension) are necessary, in our opinion, to elucidate the involvement of the K\(^+\)(Na\(^+\))/H\(^+\) exchanger in these diseases.

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