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Carrier-Mediated Residual K^+ and Na^+ Transport of Human Red Blood Cells

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Summary. Residual, i.e., (ouabain, bumetanide, and EGTA)-insensitive K^+ and Na^+ influxes as well as effluxes of human red blood cells are enhanced in isotonic solutions of low (NaCl+KCl) concentration using sucrose to maintain constant osmolarity. Various carrier models were tested to fit the experimental data of these fluxes simultaneously. The residual K^+ and Na^+ fluxes can be described on the basis of a carrier mechanism of competing substrates with modifier sites.

Key Words carrier-mediated transport · residual cation transport · K^+ and Na^+ unidirectional fluxes · human erythrocytes

Introduction

It has been known for a long time that there is an increase in unidirectional tracer K^+ efflux as well as net K^+ efflux when human red blood cells are suspended in isotonic sucrose or lactose solution of low (NaCl+KCl) concentration compared with solutions of physiological ionic composition (Davson, 1939; Wilbrandt, 1940; Wilbrandt & Schatzmann 1960; Carolin & Maizels, 1965; LaCelle & Rothstein, 1966; Bernhardt, Donath & Glaser, 1984; Jones & Knauf, 1985). An increase of the ouabain-insensitive sodium efflux in a solution of low (NaCl+KCl) concentration has also been demonstrated when the Na^+/K^+-pump is inhibited (Glaser, Bernhardt & Donath, 1980; Zade-Oppen, Adragna & Tosteson, 1988). A change in the membrane permeability induced in solutions of low (NaCl+KCl) concentration has been discussed as the reason for these increases in K^+ and Na^+ efflux. Using the Goldman flux equation (Goldman, 1943), Donlon & Rothstein (1969) calculated an increased permeability coefficient for K^+ with the change in the transmembrane potential which occurred in sucrose medium. Bernhardt et al. (1984) modified the Goldman equation taking into account the inner and outer surface potentials of the red cell membrane. It was therefore possible to explain the increase in K^+ efflux from human red blood cells in an isotonic solution of low NaCl concentration without a change in the permeability coefficient. However, it has recently been suggested that this was unlikely to be a general explanation for this effect. It was shown that by reducing the extracellular NaCl concentration (at constant KCl concentration) the transmembrane potential of both bovine and human erythrocytes changes by the same amount (Bernhardt et al., 1986). However, the same conditions which produce a large increase in K^+ efflux of human erythrocytes, do not induce a significant increase in K^+ efflux of bovine erythrocytes (Bernhardt et al., 1986; Erdmann et al., 1990).

It is well known that there are a number of specific transport pathways for K^+ and Na^+ in the red cell membrane (Dunham, Stewart & Ellory, 1980; Ellory et al., 1982; Chipperfield, 1986; Escobales & Canessa 1986; for review *see* Bernhardt, Hall & Ellory, 1988). Taking into account these different transport pathways, the effect of low (NaCl+KCl) concentration media on residual, i.e., (ouabain, bumetanide and EGTA)-insensitive K^+ and Na^+ effluxes has been reinvestigated.

In the last few years it could further be shown that the residual K^+ and Na^+ effluxes, as well as influxes of human red blood cells, increase significantly in a solution of low (NaCl+KCl) concentration (Bernhardt, Ellory and Hall, 1987; Zade-Oppen et al., 1988). Bernhardt, Hall and Ellory (1991) characterized residual K^+ and Na^+ influxes in detail. Both fluxes were linear with concentration over the range investigated (0.25–10 mM). K^+ flux measurements showed that the reduced NaCl concentration is of more importance for the enhanced residual K^+ influx than a change of transmembrane potential or intracellular pH. However, a small pH- and also volume-sensitivity could be shown (Bernhardt, et al., 1991). It was demonstrated that the KCl cotransport is not involved in the increase of residual K^+ influx (Erdmann et al., 1991), but that hydrostatic

pressure enhanced the residual K^+ influx of human erythrocytes in a solution of physiological ionic composition (Hall & Ellory, 1986). In media of low (NaCl+KCl) concentration hydrostatic pressure enhanced the residual K^+ influx synergistically (Bernhardt et al., 1991). Furthermore, a paradoxical temperature dependence of K^+ influx of human red blood cells has been found (Stewart, Ellory & Klein, 1980; Blackstock & Stewart, 1986). This effect is more pronounced in a solution of low (NaCl+KCl) concentration, the K^+ influx showing a minimum at about 281 K. These findings support the thesis that the experimental results mentioned above cannot be explained on the basis of electrodiffusion. When at a given low ionic strength a threefold decrease of the K^+ influx is expected from the Goldman flux equation, a tenfold increase was in fact observed.

It is therefore the aim of the present investigation to find out whether the observed effects of an increase of K^+ and Na^+ effluxes, as well as influxes in solution of low (NaCl+KCl) concentration, can be explained on the basis of a carrier model.

Materials and Methods

Erythrocyte Preparation

Human erythrocytes from whole blood ACD-preserves were used not later than four days after sampling (storage at 277 K). Plasma and buffy coat were removed by aspiration. The blood was washed three times by centrifugation (2,000 × *g*, 8 min) in a medium of the following composition (mM): NaCl, 145; KCl, 7.5; glucose, 10; Na_2HPO_4/NaH_2PO_4, 5.8, pH 7.4. After this, the cells were washed once in a medium which had the same composition as the flux medium (except for radioisotopes and inhibitors (*see* figure legends for details)).

Residual $^{22}Na^+$ and $^{86}Rb^+$ Efflux Measurements

The method of continuous efflux measurements was described in detail elsewhere (Bernhardt et al., 1984). Briefly, after 2-hr incubation at 310 K in the presence of $^{22}NaCl$ (about 0.5 MBq/ml) and $^{86}RbCl$ (about 5 MBq/ml), respectively, the suspension of erythrocytes (hematocrit 40%) was put into a diffusion chamber (vol. 0.1 ml) which was closed by a membrane filter (Satorius, pore size 3 μm) and then continuously flushed with nonradioactive solutions (T = 310 K) of different NaCl concentration (replacement by sucrose, 300 mosmol/liter, measured with vapor osmometer), 7.5 mM KCl, phosphate buffer (5.8 mM Na_2HPO_4/NaH_2PO_4, pH = 7.4) in the presence of ouabain (0.1 mM), bumetanide (0.1 mM) and EGTA (0.1 mM). The chamber was mounted on a slowly rotating axis to prevent sedimentation. The loss of radioactivity was detected continuously by means of scintillation counting. The rate constant was calculated as the negative slope of the linear regression line obtained by semilogarithmic plot of counts against time. Taking into account an intracellular K^+ and Na^+ concentration on average of 90 and 13 $mmol/liter_{cells}$, respectively (Marongiu, Holtmeier & von Klein-Wiesenberg, 1966), the rate constants were converted into effluxes in $mmol/(liter_{cells}\ hr)$.

Residual $^{22}Na^+$ and $^{86}Rb^+$ Influx Measurements

Since it is known that the residual K^+ influx of human erythrocytes is significantly different in fresh red blood cells compared to cells drawn from stored blood (Bernhardt et al., 1991), we used erythrocytes from stored blood for all experiments. Washed red cells were suspended at about 5% hematocrit in a total volume of 1 ml of flux medium contained in an Eppendorff 1.5-ml microcentrifuge tube. In all experiments ouabain (0.1 mM), bumetanide (0.1 mM), and EGTA (0.1 mM) were present. The cell suspension was equilibrated at the flux temperature (310 K) for 5 min after which tracer solution ([KCl+^{86}RbCl] or [(NaCl+^{22}NaCl], 7.5 mM) was added. When outside [KCl+^{86}RbCl] was varied up to 105 mM in the kinetic experiment (*see* Fig. 8), ^{86}Rb (in KCl) was added to give the appropriate final concentration. The duration of exposure of cells to isotope (i.e., the flux time) was 30 min. The isotope uptake was stopped by centrifugation at 15,000 × *g* (10 sec), and the supernatants removed by aspiration. The cells were then washed free of extracellular radioactivity by four successive resuspensions and centrifugations (15,000 × *g*, 10 sec) in ice-cold medium comprising (mM): $MgCl_2$, 107; N-morpholinopropanesulfonic acid (MOPS), 10 (pH 7.4). The cell pellet was lyzed with 0.5 ml of 0.1% (v/v) Triton X-100 and the protein precipitated by adding 0.5 ml of 5% (w/v) trichloracetic acid followed by centrifugation at 15,000 × *g* for 5 min. The activity of $^{22}Na^+$ and $^{86}Rb^+$ in the supernatant was determined in a liquid scintillation analyzer. The specific activity of the $^{22}Na^+$ and $^{86}Rb^+$ solutions was determined by counting a suitable sample of the radioactive stock solution with known chemical concentrations.

Statistical Analysis

Statistical analysis was performed with Student's *t*-test for paired and unpaired data. Experimental results represent mean values ± SD of at least four independent experiments. *P* values smaller than 0.05 were regarded as statistically significant.

Results and Discussion

Residual K^+ and Na^+ effluxes through red blood cell membranes increase in a solution of low (NaCl+KCl) concentration (NaCl replaced by sucrose, same osmolarity) compared with a solution of physiological (NaCl+KCl) concentration. The unidirectional K^+ and Na^+ effluxes as a function of extracellular (NaCl+KCl) concentration are represented in Figs. 1 and 2, respectively. In media of low (NaCl+KCl) concentration there is also a considerable enhancement of the residual K^+ and Na^+ uptake (Figs. 3 and 4, respectively). K^+ and Na^+ effluxes as well as K^+ and Na^+ influxes show a similar de-

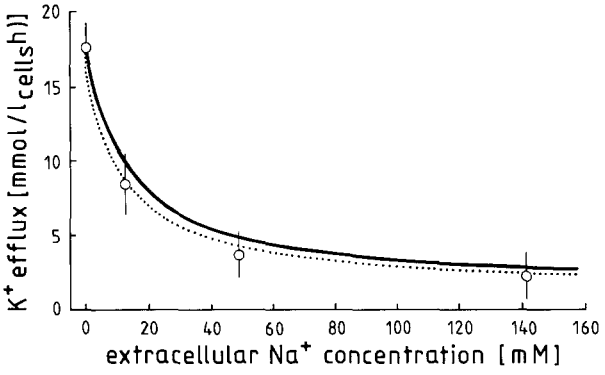

Fig. 1. The effect of extracellular Na^+ concentration on unidirectional residual K^+ efflux from human red blood cells (Na^+ replacement by sucrose; constant osmolarity, 300 mosmol/liter; constant extracellular K^+ concentration, 7.5 mM; pH 7.4; T = 310 K). Symbols represent the mean of five independent experiments ± SD The dotted line represents fluxes via the carrier model for competing substrates (Fig. 6) calculated by Eq. (18) with the numerical values of phenomenological parameters given in Eq. (29); identical to fluxes calculated by Eqs. (18, 22, 25) with $\kappa_1 = 745.67$, $\kappa_2 = 10{,}000$, $\kappa_3 = 3.10$, $K_1 = 6.447$, $K_2 = 0.021$, $K_3 = 0.0459$, $K_4 = 23.72$. The unbroken line represents flux values of the carrier model with modifier sites (Fig. 9) calculated by Eqs. (18, 25, 32) using the numerical values of parameters of the elementary steps given in Eq. (37).

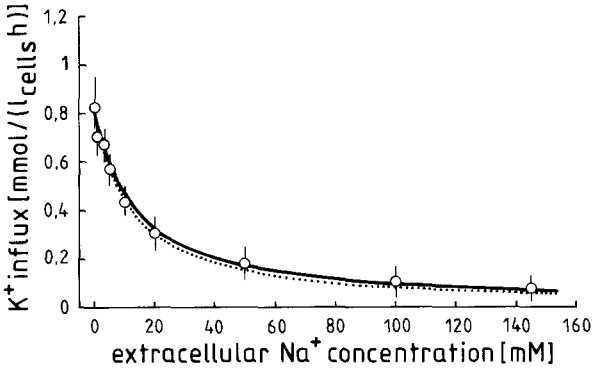

Fig. 3. The effect of extracellular Na^+ concentration on unidirectional residual K^+influx in human red blood cells. Experimental conditions, symbols and values of model parameters are the same as given in the legend to Fig. 1. Dotted line: results of the carrier model with competing substrates (Fig. 6; Eqs. (17, 20, 21)). Unbroken line: results of the carrier model with modifier sites (Fig. 9; Eqs. (17, 30, 31)).

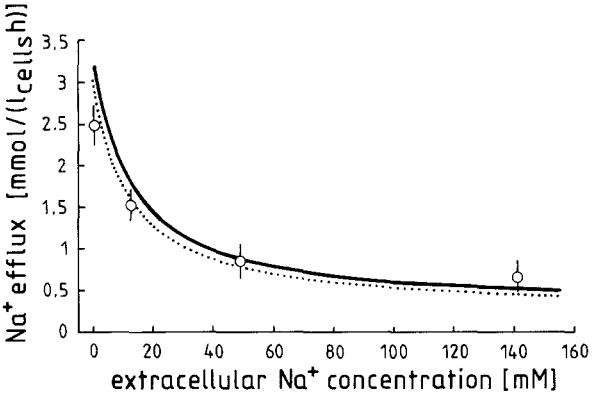

Fig. 2. The effect of extracellular Na^+ concentration on unidirectional residual Na^+ efflux from human red blood cells. Experimental conditions, symbols and values of model parameters are the same as given in the legend to Fig. 1. Dotted line: results of the carrier model with competing substrates (Fig. 6; Eqs. (19, 23, 24, 25)). Unbroken line: results of the carrier model with modifier sites (Fig. 9; Eqs. (19, 25, 33, 34)).

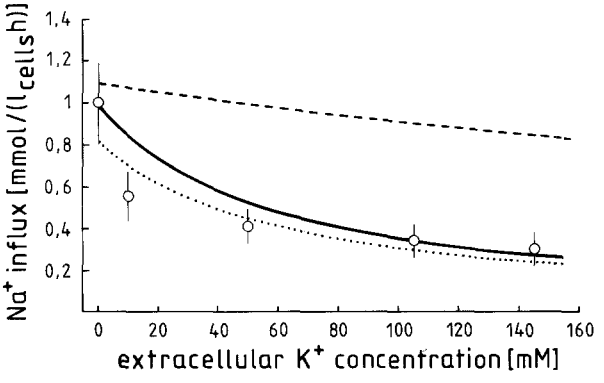

Fig. 4. The effect of extracellular K^+ concentration on unidirectional residual Na^+ influx in human red blood cells (K^+ replacement by sucrose; constant osmolarity, 300 mosmol/liter; constant extracellular Na^+ concentration, 7.5 mM; pH 7.4; T = 310 K). Symbols represent the mean of at least four independent experiments ± SD. The dotted line represents Na^+ influxes via the carrier model for competing substrates (Fig. 6) calculated by Eq. (26) with the numerical values of phenomenological parameters given in Eq. (29). Broken line: results of the carrier model with competing substrates using parameters of elementary steps (Fig. 6; Eqs. (26, 27, 28); values of the model parameters of elementary steps as given in the legend to Fig. 1). Unbroken line: results of the carrier model with modifier sites (Fig. 9; Eqs. (26, 35, 36); values of model parameters of the elementary steps as given in the legend to Fig. 1).

pendence on extracellular (NaCl+KCl) concentration. Over the higher range of (NaCl+KCl) concentration, corresponding to about 50–145 mM, there were only small changes in these four fluxes. However, below 20 mM significant increases of residual K^+ and Na^+ effluxes as well as influxes could be detected.

Flame photometric measurements showed that variations of the external K^+ and Na^+ concentrations do not change significantly the intracellular concentrations of these ions (*data not shown*). Accordingly, only the extracellular K^+ and Na^+ concentrations enter the various carrier models discussed below as variable quantities.

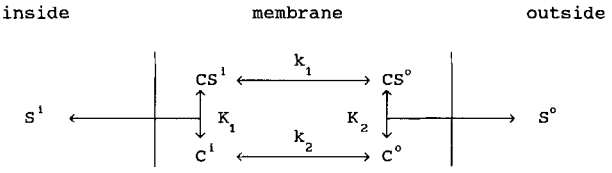

Fig. 5. Model of carrier-mediated transport (uniport mechanism). C and CS represent free and bound forms, respectively, of the carrier. k_1 and k_2 are rate constants of the translocation of C and CS, respectively, across the membrane. K_1 and K_2 are dissociation constants.

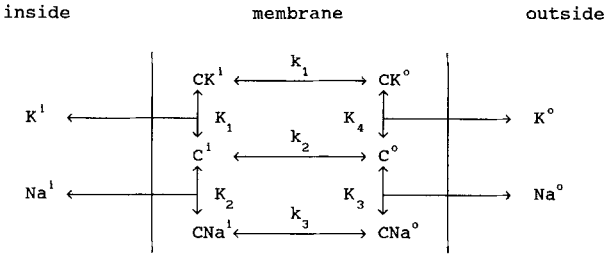

Fig. 6. Model of carrier-mediated transport for the two competing substrates K^+ and Na^+. C, and CK, CNa are free and bound forms, respectively, of the carrier. k_1, k_2, and k_3 are rate constants of translocation across the membrane of the various carrier forms. K_j are dissociation constants.

Uniport Mechanism

In a first model it was assumed that the carrier does not discriminate between K^+ and Na^+ and that the substrate concentration for the carrier is the sum of K^+ and Na^+ concentration. The transport cycle of the carrier consists of four steps: (i) association of substrate S and carrier C at the membrane interfaces, (ii) translocation of the carrier-substrate-complex CS, (iii) dissociation of CS, and (iv) back transport of free carrier C (Fig. 5). More specifically, the following assumptions are made (*cf.* Schultz, 1980):

(a) The rates of association and dissociation of the substrate are much higher than the rates of translocation. Under these conditions, the reactions at the interfaces are close to equilibrium and can be described as follows:

$$K_1 = \frac{[C^i][S^i]}{[CS^i]};\ K_2 = \frac{[C^o][S^o]}{[CS^o]} \qquad (1a,b)$$

where K_1 and K_2 are dissociation constants. The upper indices i and o denote carrier states and substrate concentrations at the inner and outer membrane surface, respectively. (b) The rate constants of translocation (k_j, k_{-j}) are independent of the direction of motion ($k_j = k_{-j}$). (c) Under steady-state conditions the distribution of the carrier at the two interfaces is time independent so that:

$$k_1([CS^o] - [CS^i]) + k_2([C^o] - [C^i]) = 0 \qquad (2)$$

(d) The carrier is confined to the membrane and its total concentration [C_t] remains constant:

$$[C_t] = [C^o] + [CS^o] + [C^i] + [CS^i] = \text{const.} \qquad (3)$$

Equations (1–3) may be solved for the concentrations of the four carrier forms ([C^o], [C^i], [CS^o], [CS^i]) in terms of substrate concentration ([S^o], [S^i]), dissociation constants (K_1, K_2), and rate constants of translocation (k_1, k_2). Calculation of the unidirectional fluxes

$$J^{io} = k_1\,[CS^i]\ \text{(efflux)};\ J^{oi} = k_1\,[CS^o]\ \text{(influx)} \qquad (4a,b)$$

yields for the uniport mechanism the following results:

(i) The efflux is determined by the ratio of k_1 and k_2. For $k_1 > k_2$, the efflux increases with increasing extracellular concentration of S ([K^+] + [Na^+]). For $k_1 < k_2$, the model describes a decreasing efflux of S under this condition, which would be in accordance with the experimental results. For $k_1 = k_2$, the unidirectional efflux is independent of the extracellular concentration of S. (ii) Independent of the ratio of k_1 and k_2 the unidirectional influx of S is described by a saturation function in dependence on [S] which is in contrast to the data shown in Figs. 3 and 4. One may conclude, therefore, that the uniport mechanism cannot give a sufficient explanation of the experimental results.

Carrier-Mediated Transport with Two Competing Substrates

For carriers which are able to discriminate between the substrates K^+ and Na^+, more extended reaction schemes have to be considered. Figure 6 shows a mechanism where the substrates compete for the same binding site of the transport system. It can be described by a similar set of equations as the uniport mechanism (*cf.* Eqs. (1–3)):

$$K_1 = \frac{[C^i][K^i]}{[CK^i]};\ K_4 = \frac{[C^o][K^o]}{[CK^o]} \qquad (5a,b)$$

$$K_2 = \frac{[C^i][Na^i]}{[CNa^i]};\ K_3 = \frac{[C^o][Na^o]}{[CNa^o]} \qquad (6a,b)$$

(mass law equilibria at the two membrane interfaces);

$$k_1([CK^o] - [CK^i]) + k_2([C^o] - [C^i]) + k_3([CNa^o] - [CNa^i]) = 0 \quad (7)$$

(steady state of carrier distribution);

$$[C_t] = [C^i] + [C^o] + [CK^i] + [CK^o] + [CNa^i] + [CNa^o] = \text{const.} \quad (8)$$

(mass conservation).
The unidirectional fluxes of K^+ and Na^+ are given by:

$$J_K^{io} = k_1 [CK^i]; \; J_{Na}^{io} = k_3 [CNa^i] \quad (9a,b)$$

$$J_K^{oi} = k_1 [CK^o]; \; J_{Na}^{oi} = k_3 [CNa^o] \quad (10a,b)$$

A combination of Eqs. (5–8) yields $[CK^i]$, $[CK^o]$, $[CNa^i]$, $[CNa^o]$ as functions of potassium and sodium concentrations of the media at the two sides of the membrane:

$$[CK^i] = \frac{1}{D}([C_t]K_2[K^i](k_1K_3[K^o] + k_2K_3K_4 + k_3K_4[Na^o])) \quad (11)$$

$$[CK^o] = \frac{1}{D}([C_t]K_3[K^o](k_1K_2[K^i] + k_2K_1K_2 + k_3K_1[Na^i])) \quad (12)$$

$$[CNa^i] = \frac{1}{D}([C_t]K_1[Na^i](k_1K_3[K^o] + k_2K_3K_4 + k_3K_4[Na^o])) \quad (13)$$

$$[CNa^o] = \frac{1}{D}([C_t]K_4[Na^o](k_1K_2[K^i] + k_2K_1K_2 + k_3K_1[Na^i])) \quad (14)$$

with

$$D = (k_1K_2[K^i] + k_2K_1K_2 + k_3K_1[Na^i])(K_3[K^o] + K_3K_4 + K_4[Na^o]) + (k_1K_3[K^o] + k_2K_3K_4 + k_3K_4[Na^o])(K_2[K^i] + K_1K_2 + K_1[Na^i]) \quad (15)$$

In order to become independent of the total carrier concentration $[C_t]$ which is unknown, modified rate constants κ_j of translocation were defined in the following way:

$$\kappa_j = k_j[C_t] \quad (16)$$

where κ_j has the flux unit mmol/(liter$_{cells}$ hr).

Since only the external K^+ and Na^+ concentrations were considered as variables, the various flux equations can be reformulated in such a way that constant terms are lumped into new parameters (phenomenological parameters). K^+ and Na^+ efflux as well as K^+ influx (Figs. 1–3) were measured in dependence on external sodium concentration $[Na^o]$. Therefore, from Eqs. (9a,b, 10a, 11–13, 15) one obtains:

$$J_K^{oi} = J_K^{oi}([Na^o]) = \frac{1}{\alpha_1 + \alpha_2[Na^o]} \quad (17)$$

$$J_K^{io} = J_K^{io}([Na^o]) = \frac{\beta_3 + [Na^o]}{\beta_1 + \beta_2[Na^o]} \quad (18)$$

$$J_{Na}^{io} = J_{Na}^{io}([Na^o]) = \frac{\gamma_3 + [Na^o]}{\gamma_1 + \gamma_2[Na^o]} \quad (19)$$

where the phenomenological parameters (α_j, β_j, γ_j) are functions of the parameters of the elementary steps (dissociation constants and rate constants of translocation):

$$\alpha_1 = \frac{(\kappa_1K_2[K^i] + \kappa_2K_1K_2 + \kappa_3K_1[Na^i])([K^o] + K_4) + (\kappa_1[K^o] + \kappa_2K_4)(K_2[K^i] + K_1K_2 + K_1[Na^i])}{\kappa_1[K^o](\kappa_1K_2[K^i] + \kappa_2K_1K_2 + \kappa_3K_1[Na^i])} \quad (20)$$

$$\alpha_2 = \frac{(\kappa_1K_2[K^i] + \kappa_2K_1K_2 + \kappa_3K_1[Na^i])K_4 + (K_2[K^i] + K_1K_2 + K_1[Na^i])\kappa_3K_4}{\kappa_1K_3[K^o](\kappa_1K_2[K^i] + \kappa_2K_1K_2 + \kappa_3K_1[Na^i])} \quad (21)$$

$$\beta_2 = \frac{(\kappa_1K_2[K^i] + \kappa_2K_1K_2 + \kappa_3K_1[Na^i]) + (K_2[K^i] + K_1K_2 + K_1[Na^i])\kappa_3}{\kappa_1\kappa_3K_2[K^i]} \quad (22)$$

$$\gamma_2 = \frac{\kappa_1K_2[K^i] + \kappa_2K_1K_2 + \kappa_3K_1[Na^i] + (K_2[K^i] + K_1K_2 + K_1[Na^i])\kappa_3}{\kappa_3^2K_1[Na^i]} \quad (23)$$

$$\gamma_3 = \frac{\kappa_1K_3[K^o] + \kappa_2K_3K_4}{\kappa_3K_4} \quad (24)$$

The phenomenological parameters in Eqs. (17–19) are not independent of each other. The following relations hold:

$$\beta_1 = \frac{\alpha_1\beta_2}{\alpha_2}; \; \beta_3 = \gamma_3; \; \gamma_1 = \frac{\alpha_1\gamma_2}{\alpha_2} \quad (25)$$

The Na^+ influx was measured in dependence on external potassium concentration $[K^o]$ at constant external sodium concentration $[Na^o]$ (Fig. 4). From Eqs. (10b, 14–16) one obtains:

$$J_{Na}^{oi} = J_{Na}^{oi}([K^o]) = \frac{1}{\delta_1 + \delta_2[K^o]} \quad (26)$$

with the phenomenological parameters:

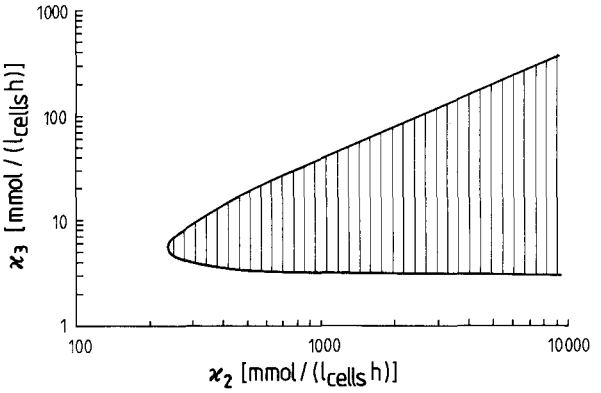

Fig. 7. Region (hatched) within the (κ_2, κ_3)-plane where positive numeric values of the parameters of the elementary steps fulfill Eqs. (20–24) with values of the phenomenological parameters α_j, β_j, γ_j given in Eq. (29).

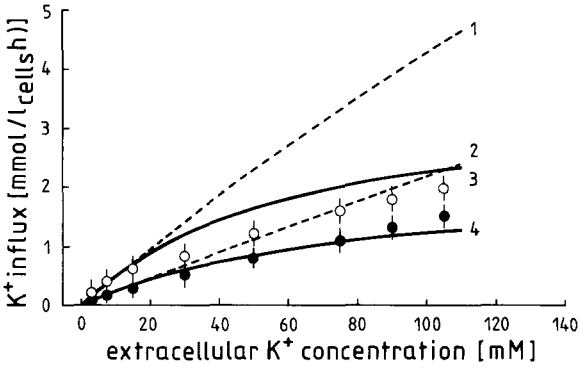

Fig. 8. The effect of extracellular K^+ concentration on unidirectional residual K^+ influx in human red blood cells measured at two fixed external Na^+ concentrations (○, $[Na^o]$ = 15 mM; ●, $[Na^o]$ = 45 mM). Symbols represent the mean of four independent experiments ± SD. Broken lines: results of the carrier model for competing substrates (Fig. 6; values of model parameters of elementary steps as given in the legend to Fig. 1; 1, $[Na^o]$ = 15 mM; 2, $[Na^o]$ = 45 mM). Unbroken lines: results of the carrier model with modifier sites (Fig. 9) calculated by the numerical values of parameters of the elementary steps given in the legend to Fig. 1 (3, $[Na^o]$ = 15 mM; 4, $[Na^o]$ = 45 mM).

$$\delta_1 = \frac{(\kappa_1 K_2[K^i] + \kappa_2 K_1 K_2 + \kappa_3 K_1[Na^i])(K_3 + [Na^o]) + (K_2[K^i] + K_1 K_2 + K_1[Na^i])(\kappa_2 K_3 + \kappa_3[Na^o])}{\kappa_3[Na^o](\kappa_1 K_2[K^i] + \kappa_2 K_1 K_2 + \kappa_3 K_1[Na^i])} \quad (27)$$

$$\delta_2 = \frac{(\kappa_1 K_2[K^i] + \kappa_2 K_1 K_2 + \kappa_3 K_1[Na^i])K_3 + \kappa_1 K_3(K_2[K^i] + K_1 K_2 + K_1[Na^i])}{\kappa_3 K_4[Na^o](\kappa_1 K_2[K^i] + \kappa_2 K_1 K_2 + \kappa_3 K_1[Na^i])} \quad (28)$$

Fitting of Eqs. (17–19, 26) to the experimental results of the respective K^+ and Na^+ fluxes shown in Figs. 1–4 were carried out by minimizing the squared residuals using a gradient method. The following numerical values of the phenomenological parameters were obtained:

$$\begin{aligned} \alpha_1 &= 1.236; \ \alpha_2 = 0.1005; \\ \beta_1 &= 9.256; \ \beta_2 = 0.7526; \ \beta_3 = 151.6; \\ \gamma_1 &= 50.30; \ \gamma_2 = 4.091; \ \gamma_3 = 151.6; \\ \delta_1 &= 1.217; \ \delta_2 = 0.0197; \end{aligned} \quad (29)$$

According to Eqs. (20–25, 27, 28) α_1, β_2, γ_2, δ_1 are expressed in the unit $liter_{cells}$ hr/mmol, α_2, δ_2 in the unit hr($liter_{cells}$/mmol)^2, and β_3, γ_3 in the unit mmol/($liter_{cells}$ hr). Using the values of phenomenological parameters given in Eq. (29), Eqs. (17–19, 26) yield the curves shown in Figs. 1–4 (dotted lines).

The present model contains seven phenomenological parameters and the same number of parameters of elementary steps. Therefore, Eqs. (20–24) and (27, 28) may be used to calculate the unknown parameters of elementary steps from the phenomenological parameters. However, it was not possible to find positive numerical values of the parameters of elementary steps which fulfill these equations. In order to confirm this finding, a direct fitting of the parameters of the elementary steps to the experimental results was tried. Also in this case the fitting was not successful if only positive values for all these parameters were taken into account.

Considering only K^+ efflux, Na^+ efflux, and K^+ influx as functions of the extracellular Na^+ concentration (Figs. 1–3), a system of five equations (20–24) with seven unknown parameters (dissociation constants and rate constants of translocation) was obtained. There are not enough equations for an unique determination of the parameters of the elementary steps. Therefore, analyzing this nonlinear equation system a two-dimensional set of solutions can be found. It is characterized in Fig. 7 in the (κ_2, κ_3)-plane as a hatched region. For given values of κ_2 and κ_3 the remaining parameters of elementary steps are fixed by Eqs. (20–24). One may conclude that there is an infinite number of combinations of positive parameters which yield the same curves as shown in Figs. 1–3 (dotted lines). Using any set of numerical values obtained from the allowed region shown in Fig. 7, one may also calculate curves for the Na^+ and K^+ influx in dependence on extracellular K^+ concentration $[K^o]$. Typical results are shown in Figs. 4 and 8 (broken lines).

It is concluded that a theoretical description of Na^+ and K^+ influxes as a function of the extracellular K^+ concentration requires other numerical values for the parameters of the elementary steps than K^+ efflux, Na^+ efflux and K^+ influx as functions of the extracellular Na^+ concentration.

In order to achieve a coherent mathematical description of all experimental data, the model of a

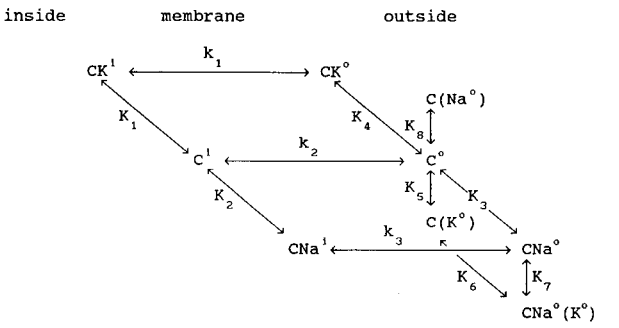

Fig. 9. Model of carrier-mediated transport with two competing substrates K^+ and Na^+ and with modifier sites. C and CK, CNa are free and bound forms, respectively, of the carrier. Substrates bound at modifier sites are indicated in brackets. k_1, k_2, and k_3 are the rate constants of translocation of C, CK, and CNa, respectively, across the membrane. K_j are dissociation constants.

competing carrier mechanism was extended. There are several possibilities for a model extension. The following simple case is considered.

Carrier-Mediated Transport for Two Competing Substrates with Modifier Sites

In addition to transfer sites which represent specific regions of transport protein molecules involved in substrate binding and translocation (*as shown* in Fig. 6), so-called modifier sites which are capable of reacting with specific modifiers of transport have been discussed for carrier-mediated transport (Dalmark, 1975; Passow, 1986). The mathematical treatment of the influence of modifier sites on the carrier model can be accomplished by an extension of the reaction scheme shown in Fig. 6. A minimal expansion of this reaction scheme by modifier sites is shown in Fig. 9. Since in the experiments only the ionic composition of the extracellular solution was varied and since the extended model should have a minimal number of new parameters, only modifier sites at the outer membrane surface were introduced. In the model without modifier sites there was a discrepancy between the experimental results of the Na^+ influx and the theoretical description using parameters of elementary steps. Therefore, modifier sites affecting mainly carrier forms loaded with sodium were introduced.

Analyzing the model with modifier sites (Fig. 9), the same phenomenological Eqs. (17–19, 25, 26) as given above for the competing carrier mechanism are obtained. However, there are modified relations between the phenomenological parameters and the parameters of the elementary steps:

$$\alpha_1 = \frac{(\kappa_1[K^i]K_2 + \kappa_2K_1K_2 + \kappa_3[Na^i]K_1)(K_4K_5 + K_4[K^o] + K_5[K^o]) + (K_1K_2K_5 + K_2K_5[K^i] + K_1K_5[Na^i])(\kappa_1[K^o] + \kappa_2K_4)}{\kappa_1[K^o]K_5(\kappa_1[K^i]K_2 + \kappa_2K_1K_2 + \kappa_3[Na^i]K_1)} \quad (30)$$

$$\alpha_2 = \frac{(\kappa_1K_2[K^i] + \kappa_2K_1K_2 + \kappa_3K_1[Na^i])(K_4K_5K_6K_8 + K_3K_4K_5K_6 + K_3K_4K_8[K^o]) + \kappa_3K_4K_5K_6K_8(K_1K_2 + K_2[K^i] + K_1[Na^i])}{\kappa_1K_3K_5K_6K_8[K^o](\kappa_1[K^i]K_2 + \kappa_2K_1K_2 + \kappa_3[Na^i]K_1)} \quad (31)$$

$$\beta_2 = \frac{\kappa_3K_5K_6K_8(K_2[K^i] + K_1K_2 + K_1[Na^i]) + (\kappa_1[K^i]K_2 + \kappa_2K_1K_2 + \kappa_3[Na^i]K_1)(K_5K_6K_8 + K_3K_5K_6 + K_3[K^o]K_8)}{\kappa_1\kappa_3[K^i]K_2K_5K_6K_8} \quad (32)$$

$$\gamma_2 = \frac{\kappa_3K_5K_6K_8(K_2[K^i] + K_1K_2 + K_1[Na^i]) + (\kappa_1[K^i]K_2 + \kappa_2K_1K_2 + \kappa_3[Na^i]K_1)(K_5K_6K_8 + K_3K_5K_6 + K_3[K^o]K_8)}{\kappa_3^2[Na^i]K_1K_5K_6K_8} \quad (33)$$

$$\gamma_3 = \frac{\kappa_1K_3[K^o] + \kappa_2K_3K_4}{\kappa_3K_4} \quad (34)$$

$$\delta_1 = \frac{(\kappa_2K_3 + \kappa_3[Na^o])(K_2K_8[K^i] + K_1K_2K_8 + K_1K_8[Na^i]) + (K_3K_8 + K_3[Na^o] + K_8[Na^o]) + (\kappa_1K_2[K^i] + \kappa_2K_1K_2 + \kappa_3K_1[Na^i])}{\kappa_3K_8[Na^o](\kappa_1K_2[K^i] + \kappa_2K_1K_2 + \kappa_3K_1[Na^i])} \quad (35)$$

$$\delta_2 = \frac{K_3(\kappa_1K_2[K^i] + \kappa_2K_1K_2 + \kappa_3K_1[Na^i])(K_5K_6 + K_4K_6 + K_4[Na^o]) + \kappa_1K_3K_5K_6(K_2[K^i] + K_1K_2 + K_1[Na^i])}{\kappa_3K_4K_5K_6[Na^o](\kappa_1K_2[K^i] + \kappa_2K_1K_2 + \kappa_3K_1[Na^i])} \quad (36)$$

Here, K_5, K_6, K_7, and K_8 are dissociation constants involving the modifier sites of the carrier mechanism which are shown in Fig. 9. Taking into account the values of the phenomenological parameters given in Eq. (29), Eqs. (30–36) represent seven equations for ten unknown parameters of the elementary steps. In contrast to the model with two competing substrates without modifier sites no attempt was made to solve this equation system to obtain regions of allowed values of parameters of elementary steps. The following numerical values of the parameters of the elementary steps were obtained by a direct fit of the Eqs. (17–19, 26, 30–36) to the experimental data of K^+ efflux, Na^+ efflux and K^+ influx depending on external Na^+ concentration (Figs. 1–3), as well as Na^+ influx depending on external K^+ concentration (Fig. 4):

$$\begin{aligned} &\kappa_1 = 21.965;\ \kappa_2 = 7518;\ \kappa_3 = 23.92;\\ &K_1 = 0.1788;\ K_2 = 0.1544;\ K_3 = 0.5357;\ K_4 = 0.8127;\\ &K_5 = 0.3513;\ K_6 = 16.72;\ K_7 = 10.965;\ K_8 = 0.0763; \end{aligned} \quad (37)$$

where the units of κ_j and K_j are mmol/(liter$_{cells}$ hr) and mM, respectively. For thermodynamic reasons the parameters fulfill the following equation:

$$K_5K_6 = K_3K_7 \tag{38}$$

The theoretical results for these four unidirectional fluxes are shown in Figs. 1–4 as unbroken lines. In Fig. 8 the resulting theoretical curves for the K^+ influx as a function of external K^+ concentration are shown as unbroken lines, from which experimental data were not taken into account at the fitting procedure. It should be pointed out that the results of model fitting depend, in this case, on the starting point in the parameter space. There are, therefore, various combinations of parameters which lead to a similar or possibly better fit of the experimental values than the parameters given in Eq. (37).

Conclusion

The central question of our investigations was to find out whether it is possible to find a model of ion transport which is able to describe the experimental results of residual unidirectional K^+ efflux, Na^+ efflux, K^+ influx, and Na^+ influx in human erythrocytes. If the various residual fluxes presented are carried by one transport system, an important criterion for the validity of a model is whether kinetic parameters (parameters of elementary steps characterizing this transporter) can be found which allow a simultaneous fit of the experimental values of all residual fluxes.

The model of a uniport mechanism (Fig. 5) is not able to describe the enhancement of the residual K^+ and Na^+ influx in media of reduced (NaCl+KCl) concentration. On the basis of the carrier model for competing substrates (Fig. 6), it is possible to describe increased effluxes as well as influxes. However, a simultaneous fit (with an unique set of parameters of elementary steps) could only be obtained for K^+ and Na^+ efflux and the K^+ influx. A minimal extension of this model by introducing modifier sites (Fig. 9) leads to a greater number of independent parameters of elementary steps which allow a simultaneous fit of K^+ and Na^+ efflux as well as influx as a function of the extracellular Na^+ or K^+ concentration. In addition, K^+ influx as a function of external K^+ concentration could also be described qualitatively by the same parameters of the extended model. The carrier models presented in this paper contain a rather high number of adjustable parameters. However, the mathematical analysis shows that the experimental data presented in Figs. 1–4 cannot be fitted by using models including only a low number of kinetic parameters. For the carrier with two competing substrates it was also not possible to find a set of positive numerical values for the seven parameters of elementary steps which could fit the experimental data. It was, therefore, necessary to extend the mathematical analysis by taking into consideration modifier sites which leads to a model with 10 adjustable kinetic parameters of elementary steps.

In the model only one modifier site was introduced for Na^+ at the outer membrane surface. It is obvious that modifier sites for K^+ as well Na^+ could exist on both sides of the membrane. However, the consideration of more than one modifier site would increase the number of adjustable parameters of elementary steps. Detailed experiments are necessary to find out how many modifier sites really occur on the carrier.

A carrier with modifier sites was also discussed as a possible mechanism for the transport of anions via band 3 (Passow, 1986). At this stage of the investigations it cannot be concluded whether there is a band 3 mediated K^+ and Na^+ transport as suggested by Jones and Knauf (1985). It is interesting to note that for a hypothetical carrier concentration of 0.02 mmol/liter$_{cells}$ (equivalent to 10^6 copies of the carrier in one cell membrane and corresponding to the band 3 concentration) and assuming a membrane thickness of 8×10^{-9} m the parameters κ_1, κ_2, and κ_3 given in Eq. (37) result in permeability coefficients $P_1 = 2.5 \times 10^{-9}$ m/sec, $P_2 = 8.4 \times 10^{-7}$ m/sec, and $P_3 = 2.7 \times 10^{-9}$ m/sec.

Possible effects of the membrane potential on the carrier transport were not taken into account in the present paper. A carrier which transports exclusively K^+ and Na^+ should be affected by the membrane potential either in its loaded or free form. However, no significant dependence of the residual K^+ and Na^+ fluxes of red blood cells on the membrane potential could be observed (Bernhardt et al., 1991). Furthermore, it has to be considered that also anions could be involved in the transport via the carrier. In this case, both the loaded and the free form of the carrier could be electroneutral.

The question arises whether there are other transport pathways in the erythrocyte membrane which might mediate the residual K^+ and Na^+ fluxes. It could be shown that the $Na^+/K^+/Cl^-$-cotransport as well as the K^+/Cl^--cotransport are not involved in the increase of the unidirectional residual K^+ and Na^+ fluxes of red blood cells in solution of low (NaCl+KCl) concentration (Bernhardt et al., 1991; Erdmann et al., 1991). Another possibility could be the voltage-activated cation channel reported by Halperin et al., (1989). This channel which is activated by increasing the transmembrane potential shows different characteristics from those reported for the enhancement of the residual K^+

and Na^+ fluxes of red blood cells in solution of low (NaCl+KCl) concentration. There is an effect of the transmembrane potential on the K^+, Na^+ and Ca^{2+} transport via the voltage-activated channel of human red blood cells. This effect is not reversible and occurs in high-potassium but not low-potassium sheep red blood cells (Halperin et al., 1989, 1990). In contrast, the increase of the residual K^+ and Na^+ fluxes of human red blood cells in solution of low (NaCl+KCl) concentration are fully reversible and there is no increase of the passive Ca^{2+} influx under these conditions (Ellory, Hall & Bernhardt, 1988; Bernhardt et al., 1991). Furthermore, the described effect occurs in low-potassium but not in high-potassium sheep red blood cells (Erdmann et al., 1991). There are still other transport pathways for monovalent cations in the red blood cell membrane (*see* e.g., Bernhardt et al., 1988). However, these pathways are specific either for K^+ or for Na^+. Thus, the residual K^+ and Na^+ fluxes through the red blood cell membrane are not mediated by one of the transport mechanisms identified so far.

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