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Voltage dependence of partial reactions of the Na^+/K^+ pump: predictions from microscopic models

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A theoretical treatment of the voltage dependence of electroneutral Na^+-Na^+ and K^+-K^+ exchange mediated by the Na^+/K^+ pump is given. The analysis is based on the Post-Albers reaction scheme in which the overall transport process is described as a sequence of conformational transitions and ion-binding and ion-release steps. The voltage dependence of the exchange rate is determined by a set of 'dielectric coefficients' reflecting the magnitude of charge translocations associated with individual reaction steps. Charge movement may result from conformational changes of the transport protein and/or from migration of ions in an access channel connecting the binding sites with the aqueous medium. It is shown that valuable mechanistic information may be obtained by studying the voltage dependence of transport rates at different (saturating and nonsaturating) ion concentrations.

Introduction

The Na^+/K^+ pump in the plasma membrane of animal cells carries out uphill transport of sodium and potassium ions at the expense of free energy of ATP hydrolysis [1–7]. According to the Post-Albers reaction cycle (Fig. 1) the pump goes through a sequence of conformational transitions and ion-binding and ion-release steps. Binding of ATP and Na^+ from the cytoplasmic side to the enzyme in conformation E_1 results in phosphorylation of the protein and 'occlusion' of sodium ($Na_3 \cdot E_1 \cdot ATP \rightarrow (Na_3)E_1\text{-}P$). By transition to conformation E_2, the ion-binding sites become exposed to the extracellular medium, and Na^+ is released. Binding of K^+ from the extracellular side leads to dephosphorylation and occlusion of K^+. After transition to conformation E_2, potassium is released to the cytoplasm.

At physiological levels of intra- and extracellular ion concentrations, the pump moves three Na^+ ions inward and two K^+ ions outward, so that the overall transport process is associated with net

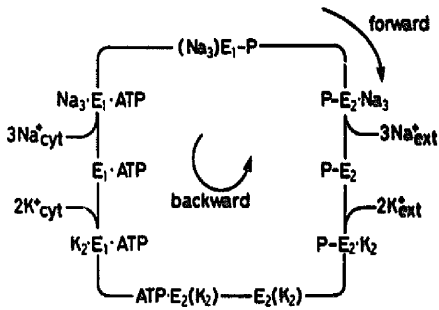

Fig. 1. Post-Albers scheme for the pumping cycle of Na^+/K^+-ATPase [8]. E_1 and E_2 are conformations of the enzyme with ion binding sites exposed to the cytoplasm and the extracellular medium, respectively. In the 'occluded' states $(Na_3)E_1$ and $E_2(K_2)$ the bound ions are unable to exchange with the aqueous phase. Dashes indicate covalent bonds and dots indicate noncovalent bonds.

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translocation of electric charge. By studying the voltage dependence of the pump current, information on the transport mechanism may be obtained [9]. For the kinetic analysis of experiments it is sometimes advantageous to chose conditions under which pump operation is restricted to part of the overall transport cycle. For instance, in the absence of K^+ and in the presence of intracellular ADP and ATP, one-for-one Na^+-Na^+ exchange may be observed [6]. Under other conditions, the pump carries out one-for-one K^+-K^+ exchange [6]. Although the overall Na^+-Na^+ exchange process is electroneutral, it may contain electrogenic reaction steps and thus may exhibit a voltage-dependent rate [10,11]. In the following, we discuss predictions on the voltage dependence of electroneutral Na^+-Na^+ and K^+-K^+ exchange based on a microscopic analysis of the transport cycle.

Voltage dependence of electroneutral exchange reactions

Na^+-Na^+ exchange

In the absence of K^+ and in the presence of intra- and extracellular Na^+ and of intracellular ADP and ATP, the Na^+/K^+ pump is engaged in electroneutral Na^+-Na^+ exchange [12–25]. Although the exchange reaction is not accompanied by net hydrolysis of ATP, it does not occur unless both ADP and ATP are present on the cytoplasmic side. The kinetic studies carried out so far are consistent with the assumption that Na^+-Na^+ exchange proceeds via the upper part of the reaction cycle of Fig. 1. This assumption is based on the observation that Na^+ is a poor substitute for K^+ in the reaction cycle of the Na^+/K^+ pump and that spontaneous dephosphorylation and transition back to state E_1 (E_2-P → E_1) is extremely slow [6]. Accordingly, under the conditions of Na^+-Na^+ exchange, the Post-Albers cycle reduces to the partial reaction sequence of Fig. 2.

According to Fig. 2, rates of the individual reaction steps are described by rate constants $a_f c_T$, p_f and l_f for transitions in forward direction and by rate constants a_b, $p_b c_D$ and l_b for transitions in backward direction; c_T, c_D and c_P are the cytoplasmic concentrations of ATP, ADP and inorganic phosphate (P_i), respectively. In the notion adopted here, the bimolecular reaction $Na_3 \cdot E_1 + ATP \rightarrow Na_3 \cdot E_1 \cdot ATP$ is described by a pseudo-monomolecular rate constant $a_f c_T$; the rate constant $p_b c_D$ is defined in an analogous way.

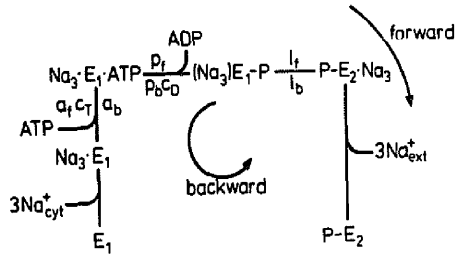

Fig. 2. Reaction sequence of the Na^+/K^+ pump under conditions of Na^+-Na^+ exchange, based on the Post-Albers cycle (Fig. 1). $a_f c_T$, p_f, l_f and a_b, $p_b c_D$, l_b are rate constants for transitions in 'forward' and 'backward' direction, respectively ('forward' and 'backward' are defined in analogy to the Na^+-K^+-exchange mode of the pump; compare Fig. 1). c_T and c_D are the cytoplasmic concentrations at ATP and ADP.

We assume that the enzyme in both conformations (E_1 and E_2) can bind up to three Na^+ ions. This means that E_j ($j = 1, 2$) can occur in the following forms: Na_3E_j, Na_2E_j, NaE_j and E_j. The three sodium sites are considered to be equivalent. We further assume that the rate constants for binding and release of Na^+ are large so that the four different forms are always in equilibrium with each other. The assumption of fast binding and release is introduced here mainly for simplicity; it can be replaced by a more refined treatment as soon as more detailed experimental data become available. If c'_N is the Na^+ concentration on the cytoplasmic side and x[A] the fraction of Na^+/K^+-ATPase present in state A, sodium binding on the cytoplasmic side is described by the following relations:

$$\frac{x[Na \cdot E_1]}{x[E_1]} = \frac{x[Na \cdot E_1 \cdot ATP]}{x[E_1 \cdot ATP]} = \frac{c'_N}{K'_{N1}} \equiv n'_1 \tag{1}$$

$$\frac{x[Na_2 \cdot E_1]}{x[Na \cdot E_1]} = \frac{x[Na_2 \cdot E_1 \cdot ATP]}{x[Na \cdot E_1 \cdot ATP]} = \frac{c'_N}{K'_{N2}} \equiv n'_2 \tag{2}$$

$$\frac{x[Na_3 \cdot E_1]}{x[Na_2 \cdot E_1]} = \frac{x[Na_3 \cdot E_1 \cdot ATP]}{x[Na_2 \cdot E_1 \cdot ATP]} = \frac{c'_N}{K'_{N3}} \equiv n'_3 \tag{3}$$

Implicit in Eqns. 1–3 is the assumption that Na^+ and ATP bind independently [26,27]. K'_{N1}, K'_{N2} and K'_{N3} are the equilibrium dissociation con-

stants of Na^+ at the cytoplasmic side. Analogous equations hold for sodium binding at the extracellular side which is described by the quantities $n_i'' \equiv c_N''/K_{Ni}''$ $(i = 1, 2, 3)$.

Under steady-state conditions the two unidirectional sodium fluxes Φ_N' and Φ_N'' become equal: $\Phi_N' = \Phi_N'' = \Phi_N$. Φ_N is the rate of Na^+-Na^+ exchange which is referred to a single pump molecule and expressed in s^{-1}. Straightforward analysis of the reaction scheme of Fig. 2 (Appendix A) leads to the following relation for Φ_N:

$$\Phi_N = \frac{3l_f p_f}{l_f + p_f c_D/K_D} \cdot \frac{\frac{c_D c_T}{K_D K_T}\rho'\rho''}{\frac{c_D}{K_D}\left(1+\frac{c_T}{K_T}\right)\rho'' + \frac{c_T}{K_T}(\rho'' + l_f/l_b)\rho'} \tag{4}$$

$$K_D \equiv p_f/p_b; \qquad K_T \equiv a_b/a_f \tag{5}$$

$$\rho' \equiv n_1' n_2' n_3'/P'; \qquad \rho'' \equiv n_1'' n_2'' n_3''/P'' \tag{6}$$

$$P' \equiv 1 + n_1' + n_1' n_2' + n_1' n_2' n_3' \tag{7}$$

$$P'' = 1 + n_1'' + n_1'' n_2'' + n_1'' n_2'' n_3'' \tag{8}$$

At low sodium and nucleotide concentrations, Φ_N becomes proportional to $c_D c_T (c_N' c_N'')^3$, as may be expected. Since the denominator of Eqn. 4 contains quadratic terms in c_D/K_D, Φ_N goes through a maximum with increasing ADP concentration c_D. This results from the fact that a high values of c_D, the reaction $(Na_3)E_1\text{-}P \rightarrow Na_3 \cdot E_1 \cdot ATP$ is strongly favoured so that state $(Na_3)E_1\text{-}P$ becomes depopulated.

In order to describe the voltage dependence of the kinetic parameters contained in Eqn. 4, we introduce the energy profile of the ion along the transport pathway [28] consisting of a series of barriers and wells (Fig. 3). According to the assumption of fast association-dissociation equilibrium, the ion binding site in state $Na_3 \cdot E_1 \cdot ATP$ is connected with the cytoplasmic side by a series of low barriers (Fig. 3); the site is separated from the extracellular medium by a high barrier.

The membrane voltage u is defined as the difference of the electrical potential ψ' in the cytoplasm and the electrical potential ψ'' in the extracellular medium and is expressed in units of $kT/e_0 \approx 25$ mV (k is the Boltzmann constant, T the absolute temperature and e_0 the elementary charge):

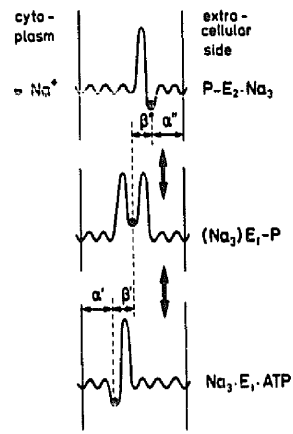

Fig. 3. Hypothetical energy profile of a Na^+ ion along the transport pathway. The ion binding sites in state $Na_3 \cdot E_1 \cdot ATP$ are connected with the cytoplasmic side by a series of low barriers, but separated from the extracellular medium by a high barrier. In the 'occluded' state $(Na_3)E_1\text{-}P$ the energy barriers on either side are high. In state $P\text{-}E_2 \cdot Na_3$ the binding sites are accessible from the extracellular phase. α', α'', β' and β'' are fractional dielectric distances.

$$u \equiv \frac{U}{kT/e_0} \equiv \frac{\psi' - \psi''}{kT/e_0} \tag{9}$$

According to Fig. 3, a fraction $\alpha' u$ of the total membrane potential u drops between the cytoplasm and the ion binding site in state E_1. This means that the equilibrium dissociation constants of Na^+ (Eqns. 1–3) become voltage dependent [29]:

$$K_{Ni}' = \tilde{K}_{Ni}' \exp(-\alpha' u) \qquad (i = 1, 2, 3) \tag{10}$$

$\tilde{K}_{Ni}'$ is the value of K_{Ni}' at zero voltage. If the potential at the cytoplasmic side is positive with respect to the external medium ($u > 0$), the equilibrium constant $1/K_{N1}'$ of sodium binding is increased by the Boltzmann factor $\exp(\alpha' u)$. The binding site acts as an 'ion well' [61], i.e., a change of electrical potential has a similar effect on the occupancy of the site as a change of external ion concentration. Implicit in Eqn. 10 is the assumption that the three sodium sites are equivalent, so that the voltage dependence of K_{N1}', K_{N2}' and

K'_{N3} is described by the same dielectric coefficient α'.

If $\alpha'' u$ is the potential at the binding site in state E_2 with respect to the extracellular medium (Fig. 3), the voltage dependence of K''_{Ni} is given by:

$$K''_{Ni} = \tilde{K}''_{Ni} \exp(\alpha'' u) \qquad (i = 1, 2, 3) \tag{11}$$

In the process $Na_3 \cdot E_1 \cdot ATP \rightarrow (Na_3)E_1\text{-}P$ the protein is phosphorylated and the bound sodium ions become occluded. This transition is, in general, associated with a conformational change whereby the bound ions may move over a certain distance. The effective dielectric distance over which the binding sites are translocated is described by a phenomenological parameter β' (Fig. 3). Accordingly, the electrostatic contribution of ions plus binding sites to the energy difference between states $Na_3 \cdot E_1 \cdot ATP$ and $(Na_3)E_1\text{-}P$ may be written as $(3 + z_L)\beta' u$, where $z_L e_0$ is the charge of the empty ligand system. If in the transition intrinsic charges of the protein (other than charged ligands) are translocated (for instance by rotation of dipolar groups), an additional energy contribution of magnitude $\eta' u$ results [30]. According to the theory of absolute reaction rates [31], the forward and backward rate constants are then given by [29]:

$$p_f = \tilde{p}_f \exp\{[(3 + z_L)\beta' + \eta']u/2\} \tag{12}$$

$$p_b = \tilde{p}_b \exp\{-[(3 + z_L)\beta' + \eta']u/2\} \tag{13}$$

In a completely analogous way one obtains for l_f and l_b:

$$l_f = \tilde{l}_f \exp\{[(3 + z_L)\beta'' + \eta'']u/2\} \tag{14}$$

$$l_b = \tilde{l}_b \exp\{-[(3 + z_L)\beta'' + \eta'']u/2\} \tag{15}$$

β'' and η'' are dielectric coefficients defined in an analogous way as β' and η'. For simplicity we assume that the rate constants a_f and a_b describing binding and release of ATP in state E_1 are voltage independent. According to Fig. 3, the dielectric coefficients are connected by the relation

$$\alpha' + \alpha'' + \beta' + \beta'' = 1 \tag{16}$$

Numerical examples for the voltage dependence of the exchange rate Φ_N are given in Figs. 4–6. For the numerical evaluation of Φ_N, the following literature values of rate constants have been introduced into Eqns. 12–15: $\tilde{l}_f = 120\ s^{-1}$, $\tilde{l}_b = 10\ s^{-1}$, $\tilde{p}_f = 170\ s^{-1}$, $\tilde{p}_b c_D = 2\ s^{-1}$ (at $c_D = 0.1$ mM). These values have been previously used for numerical simulations of the current-voltage behaviour of the Na^+/K^+ pump [29]. Assuming that the three sodium sites are identical and independent, the following relations are obtained for the equilibrium dissociation constants at the cytoplasmic side:

$$\tilde{K}'_{N1} = \tilde{K}'_N/3; \quad \tilde{K}'_{N2} = \tilde{K}'_N; \quad \tilde{K}'_{N3} = 3\tilde{K}'_N \tag{17}$$

$$\tilde{K}''_{N1} = \tilde{K}''_N/3; \quad \tilde{K}''_{N2} = \tilde{K}''_N; \quad \tilde{K}''_{N3} = 3\tilde{K}''_N \tag{18}$$

$\tilde{K}'_N$ and $\tilde{K}''_N$ are the intrinsic dissociation constants in states E_1 and E_2, respectively, for which the values $\tilde{K}'_N \approx 4$ mM and $\tilde{K}''_N \approx 100$ mM may be used [29]. The factors 1/3 and 3 are statistical coefficients describing binding equilibria in a system with multiple binding sites [32]. Relations 17 and 18 represent a strong simplification, since in reality the binding affinities of the three Na^+ sites may be different; for a further discussion of alkali-ion binding to the Na^+/K^+ pump, see Refs. 33 and 34.

For given values of the rate constants and equilibrium dissociation constants, the voltage dependence of Φ_N is determined by the dielectric coefficients α', α'',... and by the charge $z_L e_0$ of the ligand system. In agreement with previous proposals [9,11,53–55], we use a value of $z_L = -2$ in the following. Measurements of transient pump currents after an ATP-concentration jump [56,57] have given evidence that phosphorylation of the protein by ATP and formation of the occluded state is an electrically silent process, corresponding to $\beta' = 0$ (Fig. 3). Furthermore, we assume that intrinsic charge translocations other then movements of the binding sites are negligible ($\eta' = \eta'' = 0$). Under this condition voltage effects on Φ_N are determined by the voltage dependence of the rate constants l_f and l_b and of the equilibrium dissociation constants K'_N and K''_N.

In Fig. 4 the Na^+-Na^+-exchange rate Φ_N is plotted as a function of voltage for symmetrical sodium concentrations ($c'_N = c''_N = 50$ mM) and fixed concentrations of ADP (0.1 mM) and ATP

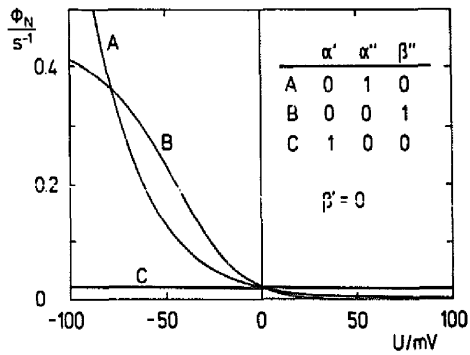

Fig. 4. Rate Φ_N of Na^+-Na^+ exchange (referred to a single pump molecule) as a function of transmembrane voltage U for different values of the dielectric coefficients α', α'' and β''. Φ_N has been calculated from Eqns. 4–18 using the following parameter values: $\tilde{I}_f = 120\ s^{-1}$, $\tilde{I}_b = 10\ s^{-1}$, $\tilde{p}_f = 270\ s^{-1}$, $\tilde{p}_b = 2 \cdot 10^4\ M^{-1} \cdot s^{-1}$, $K_T = 0.4\ \mu M$, $K'_N = 4$ mM, $K''_N = 100$ mM, $z_L = -2$, $\beta' = \eta' = \eta'' = 0$, $c_D = 0.1$ mM, $c_T = 1$ mM, $c'_N = c''_N = 50$ mM, $T = 298$ K.

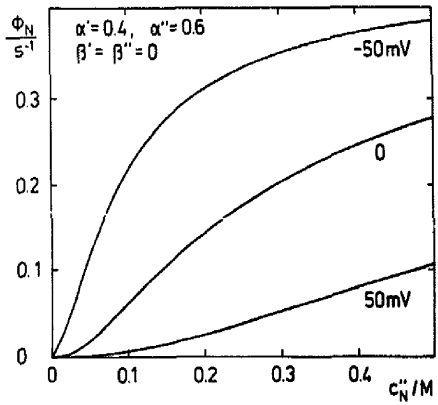

Fig. 5. Rate Φ_N of Na^+-Na^+ exchange as a function of extracellular Na^+ concentration c''_N for three different voltages (−50, 0 and 50 mV). $\alpha' = 0.4$, $\alpha'' = 0.6$, $\beta' = \beta'' = 0$, $c'_N = 100$ mM. The other parameters were the same as in Fig. 4. The three curves approach the same limiting value $\Phi_{N,max} \approx 0.45\ s^{-1}$ for $c''_N \to \infty$.

(1 mM). Three limiting cases with respect to the values of the dielectric coefficients are considered. In case A ($\alpha' = 0$, $\alpha'' = 1$, $\beta'' = 0$), the only voltage-dependent parameter is the dissociation constant K''_N of sodium at the extracellular side. Under this condition the occupancy of the extracellular sodium sites increases for inside-negative potentials as a result of a 'sodium well' effect, which in turn leads to an increase of the transport rate Φ_N. In case B ($\alpha' = \alpha'' = 0$, $\beta'' = 1$), sodium binding is voltage insensitive, and the voltage effect on Φ_N exclusively results from the voltage dependence of the rate constants I_f and I_b (Eqns. 14 and 15). In case C ($\alpha' = 1$, $\alpha'' = \beta'' = 0$), K'_N is affected by voltage U, but the dependence of Φ_N on U is weak, since under the condition $c'_N \gg K'_N$, the cytoplasmic sodium-sites remain saturated in a wide range of U.

The dependence of exchange rate Φ_N on extracellular sodium concentration c''_N is represented in Fig. 5 for three different voltages (−50, 0 and 50 mV) under the condition $c'_N = 100\ mM \gg K'_N$ for $\alpha' = 0.4$ and $\alpha'' = 0.6$. It is seen that Φ_N approaches saturation much faster at the negative voltage. This behavior again results from the voltage drop between extracellular Na^+-binding site and aqueous medium. The saturation value of Φ_N for $c''_N \to \infty$ is virtually identical for the three voltages (not shown).

In Fig. 6 the half-saturation concentration $(c''_N)_{1/2}$ is plotted as a function of voltage for different combinations of the dielectric coefficients α'' and β'' (with fixed values $\alpha' = 0.4$ and $\beta' = 0$). As may be expected, the half-saturation concentration is only weakly voltage sensitive for $\alpha'' = 0$, but becomes strongly voltage dependent for larger values of α''. Figs. 5 and 6 demonstrate that valuable information on the nature of

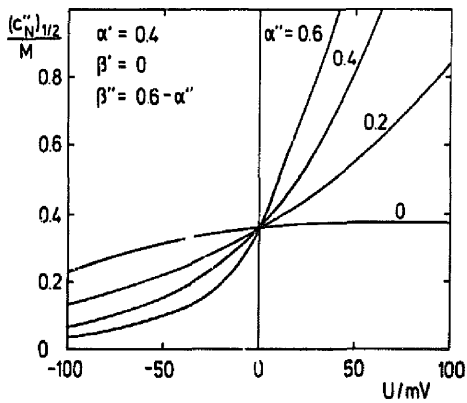

Fig. 6. Half-saturation concentration $(c''_N)_{1/2}$ of Na^+-Na^+ exchange rate Φ_N as a function of transmembrane voltage U for different combinations of the dielectric coefficients α'' and β''; α' and β' are chosen to be 0.4 and 0, respectively. The other parameters are the same as in Figs. 4 and 5.

charge-translocating steps may be obtained by studying the sodium-concentration dependence of exchange rate at different voltages.

K^+-K^+ exchange

Under sodium-free conditions, and in the presence of inorganic phosphate and ATP on the cytoplasmic side, the Na^+/K^+ pump carries out electroneutral K^+-K^+ exchange [11,35–36]. A simplified reaction scheme [38,39,47] which accounts for many experimental observations related to K^+-K^+ exchange is shown in Fig. 7. The scheme is based on the finding that the enzyme in state E_2 can be phosphorylated by inorganic phosphate [48,49] and that binding of K^+ to P-E_2 results in dephosphorylation and formation of the occluded state $E_2(K_2)$ [50,51]. ATP is known to bind to the occluded state and to increase the rate of the transition $E_2(K_2) \rightarrow K_2 \cdot E_1$ [6]. The same effect is observed with nonphosphorylating analogs of ATP [52], indicating that in the transition $K_2 \cdot E_1 \leftrightarrow E_2(K_2)$, ATP merely acts as a cofactor which modulates the magnitude of the rate constants h_f and h_b. Accordingly, ATP is omitted in the reaction sequence of Fig. 7.

Assuming that K^+ and inorganic phosphate bind independently to state E_2 [39], the potassium-binding equilibria at the intra- and extracellular side may be described by the following relations (using a similar notation as in Eqns. 1–3):

$$\frac{x[K \cdot E_1]}{x[E_1]} = \frac{c'_K}{K'_{K1}} \equiv k'_1 \tag{19}$$

$$\frac{x[K_2 \cdot E_1]}{x[K \cdot E_1]} = \frac{c'_K}{K'_{K2}} \equiv k'_2 \tag{20}$$

$$\frac{x[E_2 \cdot K]}{x[E_2]} = \frac{x[P\text{-}E_2 \cdot K]}{x[P\text{-}E_2]} = \frac{c''_K}{K''_{K1}} \equiv k''_1 \tag{21}$$

$$\frac{x[E_2 \cdot K_2]}{x[E_2 \cdot K]} = \frac{x[P\text{-}E_2 \cdot K_2]}{x[P\text{-}E_2 \cdot K]} = \frac{c''_K}{K''_{K2}} \equiv k''_2 \tag{22}$$

In a similar way as in the case of Na^+-Na^+ exchange (Appendix A), the rate Φ_K of K^+-K^+ exchange is obtained as

$$\Phi_K = \frac{2h_f q_f}{h_f + q_f c_P/K_P} \cdot \frac{\frac{c_P}{K_P}\sigma'\sigma''}{\frac{c_P}{K_P}\left(1 + \frac{K_P^*}{c_P}\right)\sigma' + (\sigma' + h_f/h_b)\sigma''} \tag{23}$$

$$K_P \equiv q_f/q_b; \qquad K_P^* = w_b/w_f \tag{24}$$

$$\sigma' \equiv k'_1 k'_2/(1 + k'_1 + k'_1 k'_2) \tag{25}$$

$$\sigma'' \equiv k''_1 k''_2/(1 + k''_1 + k''_1 k''_2) \tag{26}$$

c_P is the cytoplasmic concentration of inorganic phosphate.

Assuming that K^+ and Na^+ bind to the same sites, the equilibrium dissociation constants of K^+ at the intra- and extracellular side may be written as (compare Eqns. 10, 11, 17 and 18):

$$K'_{Ki} = \tilde{K}'_{Ki} \exp(-\alpha' u) \tag{27}$$

$$K''_{Ki} = \tilde{K}''_{Ki} \exp(\alpha'' u) \tag{28}$$

$$K'_{K1} = K'_K/2; \qquad K'_{K2} = 2K'_K \tag{29}$$

$$K''_{K1} = K''_K/2; \qquad K''_{K2} = 2K''_K \tag{30}$$

Furthermore, in a similar way as in Eqns. 12–15, the voltage dependence of the rate constants h_f and q_f (Fig. 7) may be described by:

$$h_f = \tilde{h}_f \exp\{[(2 + z_L)\gamma' + \theta']u/2\} \tag{31}$$

$$q_f = \tilde{q}_f \exp\{[(2 + z_L)\gamma'' + \theta'']u/2\} \tag{32}$$

where γ', γ'', θ' and θ'' are dielectric coefficients defined in an analogous manner as β', β'', η' and η''. The rate constants h_b and q_b of the backward transitions are obtained from Eqns. 31 and 32 by changing the sign of the exponent. For simplicity, w_f and w_b (Fig. 7) are assumed to be voltage independent. In analogy to Eqn. 16, the following relation holds:

$$\alpha' + \alpha'' - \gamma' - \gamma'' = 1 \tag{33}$$

When the pump molecule goes through the complete cycle of conformational transitions (Fig. 1), the sum of all intrinsic charge displacements must be zero. This means that

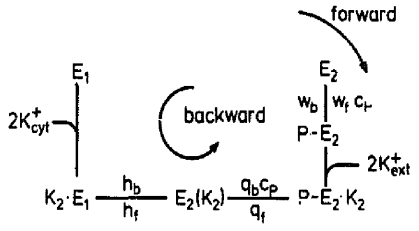

Fig. 7. Reaction sequence of the Na^+/K^+ pump under conditions of K^+-K^+ exchange. h_f, q_f, $w_f c_P$ and h_b, $q_b c_P$, w_b are rate constants for transitions in 'forward' and 'backward' direction, respectively. c_P is the cytoplasmic concentration of inorganic phosphate.

$$\eta' + \eta'' + \theta' + \theta'' = 0 \tag{34}$$

Information on the equilibrium dissociation constants of K^+ at the cytoplasmic and extracellular sites is scanty so far; in the following numerical simulation we use estimated values of $\tilde{K}'_K \approx 75$ mM and $\tilde{K}''_K \approx 0.5$ mM [2,58]. For the equilibrium constants of dissociation of inorganic phosphate from states $P\text{-}E_2 \cdot K_2$ and $P\text{-}E_2$, values of $\tilde{K}_P = \tilde{q}_f/\tilde{q}_b \approx 20$ mM [59] and $\tilde{K}^*_P = \tilde{w}_b/\tilde{w}_f \approx 1$ mM [37, 38] may be chosen (these values are based on apparent affinities and should be considered as highly tentative). The rate constants $\tilde{h}_f$, $\tilde{h}_b$ and $\tilde{q}_f$ are assigned to be $\tilde{h}_f \approx 100\ s^{-1}$ (at saturating ATP concentration) [45,47], $\tilde{h}_b \approx 300\ s^{-1}$ [58] and (as a lower limit) $\tilde{q}_f \approx 240\ s^{-1}$ [60].

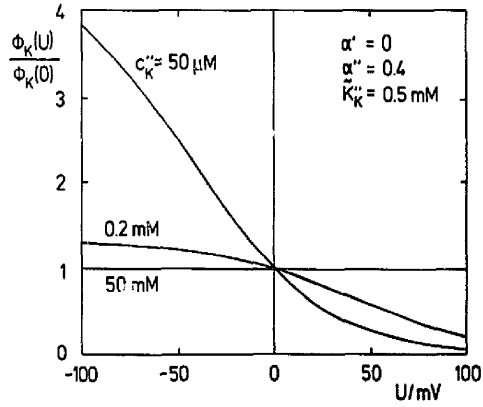

Fig. 8. Rate Φ_K of K^+-K^+ exchange as a function of voltage U for different extracellular K^+ concentrations c''_K. Φ_K is referred to the value at $U = 0$. For the calculation of Φ_K (Eqn. 23) the following parameter values have been used: $z_L = -2$, $\theta' = \theta'' = 0$, $\alpha' = 0$, $\alpha'' = 0.4$, $\tilde{K}'_K = 75$ mM, $\tilde{K}''_K = 0.5$ mM, $\tilde{K}_P = 20$ mM, $\tilde{K}^*_P = 1$ mM, $\tilde{h}_f = 100\ s^{-1}$, $\tilde{h}_b = 300\ s^{-1}$, $\tilde{q}_f = 240\ s^{-1}$, $c''_K = 50$ mM, $c_P = 1$ mM, $T = 298$ K.

According to the assumptions discussed above in the context of Na^+-Na^+ exchange, z_L, θ' and θ'' are assigned to be $z_L = -2$ and $\theta' = \theta'' = 0$. This means that the rate constants h_f, h_b, q_f and q_b (Eqns. 31 and 32) become voltage independent. A voltage effect on the exchange rate Φ_K is then only possible if α' and/or α'' have non-zero values. In Fig. 8 the voltage dependence of Φ_K is represented under the condition $\alpha' = 0$, $\alpha'' = 0.4$ for different extracellular K^+ concentrations c''_K. A pronounced voltage dependence is predicted for nonsaturating K^+ concentration ($c''_K = 50\ \mu M$), whereas the voltage effect on Φ_K virtually vanishes for $c''_K \gg \tilde{K}''$. This means that an intrinsic voltage dependence of K^+-K^+ exchange resulting from non-zero values of α' or α'' may remain undetected unless experiments are carried out in a wide range of K^+ concentrations.

Comparison with experimental results

Most studies of electroneutral Na^+-Na^+ and K^+-K^+ exchange have been carried out so far at constant membrane potential [12–25,35–46]. Milanick and Hoffman [10] recently reported that Na^+-Na^+ exchange in erythrocytes is virtually voltage-independent between $U = -50$ mV and $U = +100$ mV. This result could mean that transitions between states $Na_3 \cdot E_1 \cdot ATP$, $(Na_3)E_1\text{-}P$ and $P\text{-}E_2 \cdot Na_3$ (Fig. 2) are voltage insensitive ($\beta' \approx 0$, $\beta'' \approx 0$) and that ion-well effects are obscured by saturation. A more detailed analysis of voltage effects on Na^+-Na^+ exchange requires experiments at sub-saturating Na^+ concentrations.

Using reconstituted lipid vesicles, Goldshlegger et al. [11] studied $(ATP + P_i)$-activated exchange of Rb^+ (a K^+ congener) and found that the exchange rate was insensitive to a variation of membrane potential between 0 and −155 mV (vesicle-interior negative). Since these experiments have been done at saturating extracellular (intravesicular) Rb^+ concentrations (140–285 mM), it cannot be excluded that binding and release of Rb^+ at the extracellular side are voltage dependent (corresponding to $\alpha'' > 0$). On the other hand, since the cytoplasmic (extravesicular) Rb^+ concentrations (1–25 mM) were below saturation, the absence of

a voltage effect on the exchange rate indicates that the dielectric distance between cytoplasmic binding sites and aqueous medium is small ($\alpha' \approx 0$).

Discussion

By studying partial reactions of the Na^+/K^+ pump, such as Na^+-Na^+ or K^+-K^+ exchange, information on the pumping mechanism may be obtained. The voltage dependence of electroneutral Na^+-Na^+ and K^+-K^+ exchange can be described on the basis of reaction kinetic models. Voltage effects on the exchange rate are predicted when at least one of the individual reaction steps is associated with translocation of electric charge. Charge movements may result from conformational changes of the transport protein and/or from the migration of ions in an access channel connecting the binding sites with the aqueous medium. The fact that more than one transport step may be electrogenic usually complicates the analysis of voltage-dependent transport rates. On the other hand, the theoretical treatment given above demonstrates that valuable mechanistic information may be obtained by studying the voltage dependence of transport rates in a wide range of intra- and extracellular ion concentrations. This is illustrated by Fig. 6 in which the half-saturation concentration of extracellular sodium for Na^+-Na^+ exchange is shown as a function of voltage. While, in general, both the half-saturation concentration as well as the maximum transport rate Φ_{max} depend on voltage U, the influence of U on Φ_{max} may be small when the overall voltage dependence of transport rate is dominated by ion-well effects.

We have restricted the foregoing analysis to a treatment of electroneutral Na^+-Na^+ and K^+-K^+ exchange. A similar analysis is possible for other partial reactions of the Na^+/K^+ pump, such as electrogenic Na^+-Na^+ exchange driven by net ATP hydrolysis [62–66].

Appendix A

Derivation of Eqn. 4

In order to derive the rate Φ_N of Na^+-Na^+ exchange, we assume that sodium is present on the cytoplasmic side in the pure isotopic form 'Na' (concentration c_N') and on the extracellular side in the pure isotopic form 'Nb' (concentration c_N''). We denote the fraction of pump molecules in state A be $x[A]$ and introduce the following abbreviations:

$$x_1 \equiv x[E_1] + x[Na \cdot E_1] + x[Na_2 \cdot E_1] + x[Na_3 \cdot E_1] \quad \text{(A-1)}$$

$$x_2 \equiv x[E_1 \cdot ATP] + \ldots + x[Na_3 \cdot E_1 \cdot ATP] \quad \text{(A-2)}$$

$$x_3 \equiv x[(Na_3)E_1\text{-}P]; \qquad x_4 \equiv x[(Nb_3)E_1\text{-}P] \quad \text{(A-3)}$$

$$x_5 \equiv x[P\text{-}E_2] + x[P\text{-}E_2 \cdot Nb] + x[P\text{-}E_2 \cdot Nb_2] + x[P\text{-}E_2 \cdot Nb_3] \quad \text{(A-4)}$$

$$x_1 + x_2 + x_3 + x_4 + x_5 = 1 \quad \text{(A-5)}$$

Species such as $P\text{-}E_2 \cdot Na$ have not to be considered, since binding and release of sodium have been assumed to be always in equilibrium. Introducing the quantity $P' \equiv 1 + n_1' + n_1'n_2' + n_1'n_2'n_3'$, Eqns. 1–3, A-1 and A-2 yield the following relations:

$$x[E_1] = x_1/P'; \qquad x[E_1 \cdot ATP] = x_2/P' \quad \text{(A-6)}$$

$$x[Na_3 \cdot E_1 \cdot ATP] = n_1'n_2'n_3'x_2/P';$$

$$x[P\text{-}E_2 \cdot Nb_3] = n_1''n_2''n_3''x_5/P'' \quad \text{(A-7)}$$

Under the condition of stationary Na^+-Na^+ exchange, net phosphorylation does not occur. This leads to the equilibrium condition

$$\frac{x[E_1 \cdot ATP]}{x[E_1]} = \frac{x_2}{x_1} = \frac{a_f c_T}{a_b} \quad \text{(A-8)}$$

Furthermore, in the stationary state the following relations hold ($\dot{x}_i$ denotes the time derivative of x_i):

$$\dot{x}_3 = p_f x[Na_3 \cdot E_1 \cdot ATP] - (p_b c_D + l_f)x_3 = 0 \quad \text{(A-9)}$$

$$\dot{x}_4 = i_b x[i^*\text{-}E_2 \cdot Nb_3] - (p_b c_D + l_f)x_4 = 0 \quad \text{(A-10)}$$

$$\frac{x_3 + x_4}{x[Na_3 \cdot E_1 \cdot ATP]} = \frac{p_f}{p_b c_D} \quad \text{(A-11)}$$

The exchange rate Φ_N may be obtained from the relation

$$\Phi_N = 3l_f x[(Na_3)E_1\text{-}P] = 3l_f x_3 \quad \text{(A-12)}$$

Evaluation of x_3, using Eqns. A-5 and A-7–A-11, yields Eqn. 4.

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