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Topical Review

Electrogenic Properties of the Na,K Pump

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Introduction

The sodium-potassium 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 (Skou, 1975; Robinson & Flashner, 1979; Cantley, 1981; Schuurmans Stekhoven & Bonting, 1981; Jørgensen, 1982; Glynn, 1985; Kaplan, 1985; Stein, 1986). In its normal mode of operation, the pump performs a cycle of conformational transitions and ion-binding and ion-release steps. Spectroscopic studies as well as proteolysis experiments indicate that the enzyme can assume two principal conformations designated E_1 and E_2. Form E_1 has the ion-binding sites facing the cytoplasm and is stabilized by Na^+; form E_2 has the ion-binding sites facing the extracellular medium and is stabilized by K^+. From enzymatic and transport studies the reaction cycle has been proposed as represented in Fig. 1 (Albers, 1967; Post, Hegyvary & Kume, 1972; Cantley et al., 1984; Jørgensen & Andersen, 1988). When the protein is phosphorylated in state E_1 by ATP, Na^+ becomes "occluded," i.e., trapped inside the protein ($Na_3 \cdot E_1 \cdot ATP \rightarrow (Na_3)E_1 - P$). After transition to conformation E_2, Na^+ is released and K^+ is bound. This leads to dephosphorylation of the protein and occlusion of K^+. The original state is restored by transition to conformation E_1 and release of K^+ to the cytoplasmic side. Each of these partial reactions was identified with its appertaining "states" of the pump molecule. In the Table an overview of published rate constants is given which are able to fit at least the pumping activities of Na,K-ATPase of a purified microsomal membrane preparation of rabbit kidney (Stürmer et al., 1989).

Key Words Na,K-ATPase · electrogenic ion pumps · voltage dependence · ion flux · pump current

Since (under physiological conditions) in a single turnover three Na^+ ions are moved outward and two K^+ inward, the transport process is associated with the translocation of net charge. The electrogenic nature of the Na,K pump has interesting consequences. On the one hand, the pump acts as current generator and contributes to the membrane potential of the cell. On the other hand, the transport rate becomes a function of the membrane potential. Experimental findings confirm this proposal (Slayman, 1982; Blaustein & Lieberman, 1984; Gadsby, 1984; DeWeer et al., 1988*b*; Läuger & Apell, 1989). Before the recent investigation of the influence of voltage on different partial reactions, the overall effect of electrogenicity was observed in terms of voltage-current dependence of the Na,K pump. The influence of membrane voltage on the pumping current has been determined in nerve membranes (Hodgin & Keynes, 1955; Thomas, 1972; DeWeer, Gadsby & Rakowski, 1986; Gadsby, Rakowski & DeWeer, 1986; Rakowski, Gadsby & DeWeer, 1989), heart (Eisner & Lederer, 1980; Glitsch, 1982), muscle (Beaugé & Sjodin, 1976; Lederer & Nelson, 1984), red blood cells (Milanick & Hoffman, 1986) and in vesicles containing reconstituted Na,K pump (Goldschleger et al., 1987; Apell & Bersch, 1988). Of particular interest is the question: in which step (or steps) of the transport cycle is charge translocated?

The Voltage Dependence can be Described Theoretically

In principle, any step in the pumping cycle may involve charge translocation and therefore induce effects onto the transmembrane electrical field and conversely may be affected by the electrical field. A microscopic model has been discussed (Läuger & Apell, 1986) to reveal interactions between reaction steps of the pumping cycle and the electric field.

Table. Estimated values of kinetic parameters of the reaction scheme of Fig. 1, adapted from Stürmer et al. (1989)[a]

Transition	Parameter	Value
$3Na + E_1 \cdot ATP \rightleftarrows Na_3 \cdot E_1 \cdot ATP$	K'_N	4 mM
$3Na + E_2 - P \rightleftarrows Na_3 \cdot E_2 - P$	K''_N	100 mM
$2K + E_1 \cdot ATP \rightleftarrows K_2 \cdot E_1 \cdot ATP$	K'_K	8 mM
$2K + E_2 - P \rightleftarrows K_2 \cdot E_2 - P$	K''_K	2 mM
$Na_3 \cdot E_1 \cdot ATP \rightarrow (Na_3)E_1 - P$	p_f	180 sec^{-1}
$(Na_3)E_1 - P \rightarrow Na_3 \cdot E_1 \cdot ATP$	p_b	2×10^4 M^{-1} sec^{-1}
$(Na_3)E_1 - P \rightarrow Na_3 \cdot E_2 - P$	l_f	20 sec^{-1}
$Na_3 \cdot E_2 - P \rightarrow (Na_3)E_1 - P$	l_b	2 sec^{-1}
$K_2 \cdot E_2 - P \rightarrow (K_2)E_2$	q_f	1000 sec^{-1}
$(K_2)E_2 \rightarrow K_2 \cdot E_2 - P$	q_b	2×10^5 M^{-1} sec^{-1}
$(K_2)E_2 + ATP \rightarrow (K_2)E_2 \cdot ATP$	s_f	10^6 M^{-1} sec^{-1}
$(K_2)E_2 \cdot ATP \rightarrow (K_2)E_2 + ATP$	s_b	100 sec^{-1}
$(K_2)E_2 \cdot ATP \rightarrow K_2 \cdot E_1 \cdot ATP$	k_f	50 sec^{-1}
$K_2 \cdot E_1 \cdot ATP \rightarrow (K_2)E_2 \cdot ATP$	k_b	50 sec^{-1}
$(K_2)E_2 \rightarrow 2K + E_1$	h_f	0.3 sec^{-1}
$2K + E_1 \rightarrow (K_2)E_2$	h_b	300 sec^{-1}

[a] $T = 20°C$.

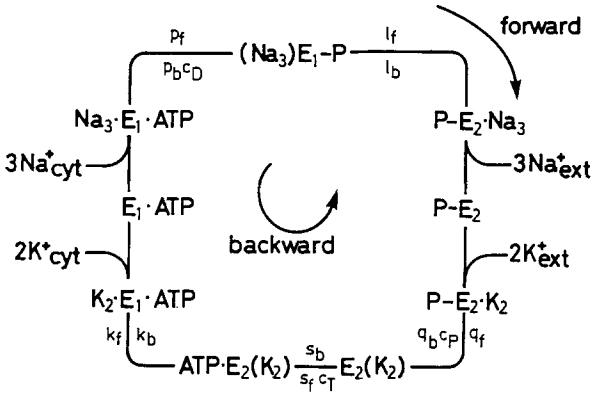

Fig. 1. Albers-Post scheme for the pumping cycle of Na,K-ATPase (adapted from Cantley et al., 1984). E_1 and E_2 are conformations of the enzyme with ion-binding sites exposed to the cytoplasm and the extracellular medium, respectively. In the "occluded" states $(Na_3)E_1 - P$ and $E_2(K_2)$ the bound ions are unable to exchange with the aqueous phase. Dashed indicate covalent bonds and dots indicate noncovalent bonds. p_f, l_f, q_f, $s_f c_T$, k_f and $p_b c_D$, k_b, s_b, $q_b c_P$, l_b are rate constants for transitions in forward and backward direction, respectively. c_T, c_D and c_P are cytoplasmic concentrations of ATP, ADP and P_i (inorganic phosphate)

As shown in Fig. 1, the pumping cycle can be divided in a series of reaction steps which describe ion binding and dissociation and transitions into different states of the pump. The binding and association of the Na^+ and K^+ ions are assumed to be fast compared to the other steps of the cycle. Therefore, the interaction of the Na,K pump with the aqueous ion compartments is described by equilibrium dissociation constants. Since up to now no direct experimental information on binding rate constants is accessible, this assumption was chosen for simplicity and can be replaced by a more refined treatment when more details become available. The other partial reactions of the pumping cycle can be treated as monomolecular or pseudo-monomolecular reactions with rate constants as shown in Fig. 1 (e.g., p_f, $p_b c_D$).

In order to describe the voltage dependence of the kinetic parameters an energy profile of the ion along the transport pathway is introduced (Parlin & Eyring, 1954). The energy profile consists of a series of barriers and wells (Fig. 2); it reflects the interaction of the ion with the protein in a particular conformation (Warshel & Russel, 1984). According to the assumption of a fast association-dissociation equilibrium, the ion-binding site in state $Na_3 \cdot E_1 \cdot ATP$ is connected with the cytoplasmic solution (or the cytoplasmic access channel) by a series of low barriers (Fig. 2); the site is separated from the extracellular medium by a high barrier.

If an electrical potential difference, u, exists between the cytoplasm and the extracellular solution, a fraction $\alpha' u$ drops between the cytoplasm and the binding site (Fig. 2). The dielectric coefficient α' ($0 \leq \alpha' \leq 1$) is a (dimensionless) quantity and depends on the location of the binding site within the protein as well as on the dielectric properties of the protein and the surrounding medium. As the potential energy of an ion in the binding site is modified by the presence of a voltage u, the equilibrium dissociation constants K'_{N1}, K'_{N2} and K'_{N3} become voltage dependent

$$K'_{Ni} = \tilde{K}'_{Ni} \exp(-\alpha' u) \qquad (1)$$

where $\tilde{K}'_{Ni}$ is the value of K'_{Ni} at zero voltage. 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 $kT/e_o = 25$ mV (k is the Boltzmann constant, T the absolute temperature and e_o the elementary charge). The dielectric coefficient α' is assumed to be the same for the three sodium-binding sites. If the potential of the cytoplasm is positive with respect to the external medium ($u > 0$), the equilibrium constant $1/K'_{Ni}$ of sodium binding is increased by the Boltzmann factor $\exp(\alpha' u)$. The binding site acts as an "ion well" (Mitchell & Moyle, 1974), i.e., a change of electrical potential has a similar effect on the occupancy of the site as a change of external concentration. Assuming that the three sodium sites are equivalent and that potassium binds to the same sites on E_1 as sodium, analogous expressions (with the same dielectric coefficient α') are obtained for K'_{N2}, K'_{N3}, K'_{K1} and K'_{K2}. If $\alpha'' u$ is the potential at the binding site in state E_2 with respect to the extracellular medium (Fig. 2), the voltage dependence of K''_{K1} is given by

$$K''_{K1} = \tilde{K}_{K1} \exp(\alpha'' u). \tag{2}$$

Analogous expressions hold for K''_{K2}, K''_{N1}, K''_{N2} and K''_{N3}.

In the process $Na_3 \cdot E_1 \cdot ATP \rightarrow (Na_3)E_1 - P$ 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 together with the ligand groups move over a certain distance. The effective dielectric distance over which the binding sites are translocated is described by a phenomenological parameter, β' (Fig. 2). The electrostatic contribution of ions plus binding sites to the energy difference between states $Na_3 \cdot E_1 \cdot ATP$ and $(Na_3)E_1 - P$ may be written as $(3 + z_L)\beta' u$ where $z_L e_o$ is the charge of the empty ligand system; the energy is expressed in units of kT. The translocation of intrinsic charges of the protein (other than charged ligands) would produce an additional energy contribution as shown previously (Läuger, 1984) and can be included into the model (Läuger & Apell, 1986). However, experimental findings show that these effects are negligible.

It is assumed, as an approximation, that the process $Na_3 \cdot E_1 \cdot ATP \rightarrow (Na_3)E_1 - P$ takes place in a single step, which may be described as a transition over a symmetrical, narrow activation barrier (Läuger, 1984). According to the theory of absolute reaction rates (Glasstone, Laidler & Eyring, 1941), the forward and backward rate constants are then given by

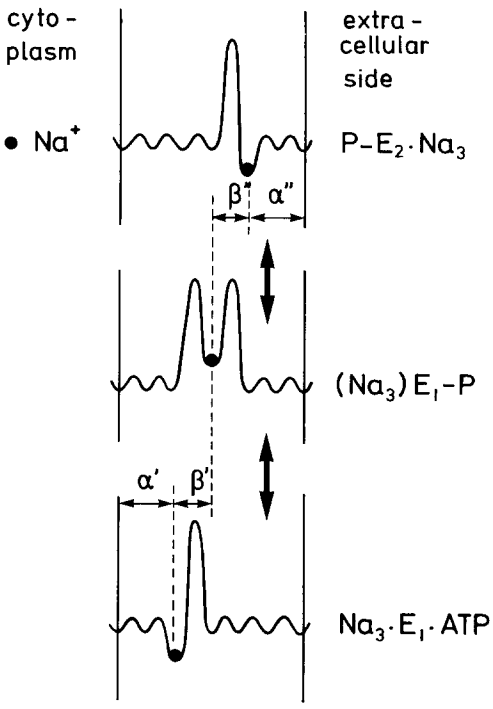

Fig. 2. Hypothetical energy profile of a Na^+ ion along the transport pathway. The ion-binding sites in state $Na_3 \cdot E_1 \cdot ATP$ are connected with the cytoplasmic side by a series of low barriers, but separated from the extracellular medium by a high barrier. In the "occluded" state $(Na_3)E_1 - P$ the energy barriers on both sides are high. In state $P - E_2 \cdot Na_3$ the binding sites are accessible from the extracellular phase. α', α'', β' and β'' are fractional dielectric distances

$$p_f = \tilde{p}_f \exp\{(3 + z_L)\beta' u/2\} \tag{3}$$
$$p_b = \tilde{p}_b \exp\{-(3 + z_L)\beta' u/2\}. \tag{4}$$

In a completely analogous way one obtains for the other forward rate constants

$$l_f = \tilde{l}_f \exp\{(3 + z_L)\beta'' u/2\} \tag{5}$$
$$q_f = \tilde{q}_f \exp\{(2 + z_L)\gamma' u/2\} \tag{6}$$
$$k_f = \tilde{k}_f \exp\{(2 + z_L)\gamma' u/2\} \tag{7}$$

where β'', γ' and γ'' are dielectric coefficients defined in an analogous way to β'. The corresponding backward rate constants are obtained from Eqs. (5)–(7) by changing the sign of the exponent. For simplicity we have assumed that the rate constants s_f and s_b describing binding and release of ATP in state $E_2(K_2)$ are voltage independent.

In the (hypothetical) process $Na^+_{cyt} + E_1 \cdot ATP \rightarrow Na \cdot E_1 \cdot ATP \rightarrow (Na)E_1 - P \rightarrow P - E_2 \cdot Na \rightarrow P - E_2 + Na^+_{ext}$ a sodium ion is translocated from the cytoplasm to the extracellular medium. The change of electrostatic energy associated with this process is the sum of the contributions of all single

steps and is equal to (in units of kT): $-(\alpha' + \beta' + \beta'' + \alpha'')u$ (Fig. 2). This sum must be equal to $-u$, the electrogenic energy required for the translocation of a univalent cation across the membrane. Therefore, the following relation must hold:

$$\alpha' + \alpha'' + \beta' + \beta'' = 1. \tag{8}$$

A similar argument applies to the translocation of a potassium ion in the sequence $K^+_{ext} + P - E_2 \rightarrow \cdots \rightarrow E_1 \cdot \mathrm{ATP} + K^+_{cyt}$, yielding

$$\gamma' + \gamma'' - \alpha' - \alpha'' = -1. \tag{9}$$

According to Eqs. (8) and (9) only four of a total of six dielectric coefficients α', α'', . . . are independent. As shown below, the dielectric coefficients will be used to identify and characterize the electrogenicity of the partial reactions of the pumping cycle.

Theoretical Methods for the Analysis of Time-Dependent Pump Currents

The operation of an ion pump may be generally described by the reaction scheme in which it is assumed that the pump goes through a cycle of conformational transitions and ion-binding and ion-release steps

$$P_1 \underset{k_2''}{\overset{k_1'}{\rightleftarrows}} P_2 \underset{k_3''}{\overset{k_2'}{\rightleftarrows}} P_3 \ldots P_n \underset{k_1''}{\overset{k_n'}{\rightleftarrows}} P_1. \tag{10}$$

This reaction cycle represents a minimal model, since branched pathways are not considered. The transition rate constants k_i' and k_i'' are, in general, pseudomonomolecular rate constants, which may contain concentrations such as ion or nucleotide concentrations. It is considered an ensemble of N pump molecules, and the fraction of pumps that are in state P_i at time t are denoted by $x_i(t)$

$$\sum_{i=1}^{n} x_i(t) = 1. \tag{11}$$

In a relaxation experiment, the system at time zero is present in a steady state, which is described by rate constants $\bar{k}_i'$, $\bar{k}_i''$ and by initial conditions $x_i = x_i(0)$. By an external perturbation, some of the rate constants are instantaneously shifted to new values k_i', k_i''. Thereafter, the system evolves toward a new stationary state with $x_i = x_i(\infty)$. The net forward rate of transitions between states P_i and P_{i+1}, referred to a single pump molecule, is given by

$$\Phi_i = k_i' x_i - k_{i+1}'' x_{i+1}. \tag{12}$$

The rate of change of x_i may then be written as

$$\frac{dx_i}{dt} = \Phi_{i-1} - \Phi_i. \tag{13}$$

Equations (11)–(13) have the formal solution (Läuger et al., 1981)

$$x_i(t) = \sum_{j=1}^{n-1} a_{ij} \exp(-t/\tau_j) + x_i(\infty). \tag{14}$$

The $n - 1$ time constants τ_j and the "amplitudes" a_{ij} are functions of the rate constants k_i' and k_i''.

In the following an experiment is considered in which a membrane with embedded ion pumps is interposed between two aqueous electrolyte solutions. Pump activation by a sudden perturbation such as a voltage or concentration jump leads, in general, to a time-dependent current $I_p(t)$ in the external measuring circuit. I_p is an average current which results from the summation of individual charge translocations in the pump molecules associated with transitions between states of the reaction cycle. The contribution of transition $P_i \rightarrow P_{i+1}$ is given by $\alpha_i e_o$, where e_o is the elementary charge and α_i the previously introduced dielectric coefficient of the transition. The meaning of α_i is simple in the limiting case of a membrane consisting of a homogeneous dielectric layer of thickness d. When in a given transition ν elementary charges are translocated over a distance a_i, the dielectric coefficient α_i is equal to $\nu a_i/d$. In general, however, the coefficients α_i depend on the geometry and the dielectric properties of the transport protein in states P_i and P_{i+1}.

Since Φ_i is the net transition rate, the contribution of the reaction $P_i \rightleftarrows P_{i+1}$ to the total current is equal to $\alpha_i e_o \Phi_i$. For an ensemble of N pump molecules, the total current is given by (Läuger et al., 1981)

$$I_p(t) = e_o N \sum_{i=1}^{n} \alpha_i \Phi_i(t). \tag{15}$$

This equation represents the basis for the microscopic interpretation of transient currents; it is valid not only for the cyclic process shown above, but for any (arbitrarily branched) reaction scheme, provided that the summation is carried out over all individual transitions.

In the steady state, all rates Φ_i are identical and equal to the stationary turnover rate Φ_s, so that the

stationary current assumes the form

$$I_{p,s} = e_o m N \Phi_s . \tag{16}$$

m is the number of elementary charges translocated across the membrane in a single turnover of the pump. This means that the following relation must hold:

$$\sum_{i=1}^{n} \alpha_i = m. \tag{17}$$

On the basis of Eqs. (12), (14) and (15) numerical simulations can be performed to determine steady-state currents (Läuger & Apell, 1986) and voltage or concentration jump experiments (Apell, Borlinghaus & Läuger, 1987; Borlinghaus & Apell, 1988; Läuger & Apell, 1988*a*,*b*).

To Gain Information about the Voltage Dependence of the Na,K-ATPase, Different Experimental Approaches are Used

The influence of membrane potential is investigated by a series of different methods whose applications, advantages and disadvantages shall be illustrated briefly.

Whole-Cell Clamp Method

The method of clamping cells to learn about the Na,K pump has been used with squid giant axons for more than 30 years (for review *see* DeWeer et al., 1988*a*). The development of the "whole cell patch clamp" technique (Hamill et al., 1981) with wide-tipped pipettes, which allow the control of the membrane voltage and the exchange of intracellular solutions (Soejima & Noma, 1984; Jauch, Petersen & Läuger, 1986) expanded the range of cells accessible to investigation.

A typical experiment is shown in Fig. 3*A*. When the pipette contacts the cell a gigaohm seal can be formed by slight suction, and after a stronger suction the membrane under the pipette is ruptured (Hamill et al., 1981). Then external and internal solutions are exchanged for media containing compounds that minimize the currents flowing through K^+, Na^+ and Ca^{2+} channels (Gadsby, Kimura & Noma, 1985). Now the transmembrane current I_{tot} can be measured at different membrane potentials U. Then the cells will be incubated with cardiotonic steroids like ouabain, and the experiment is repeated to measure current I_{ou}. The difference of

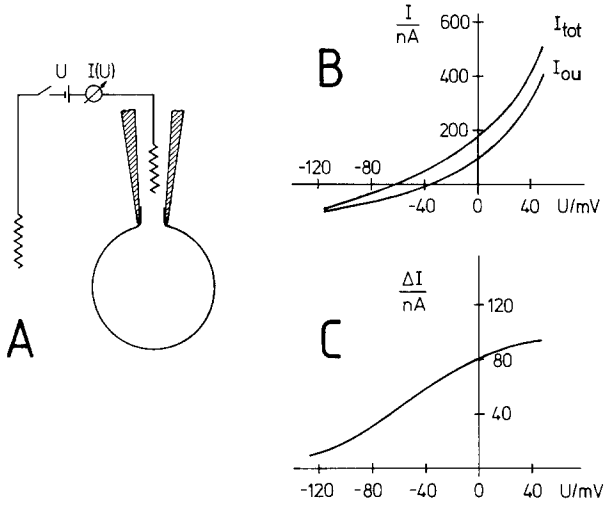

Fig. 3. Schematic presentation of the whole-cell clamp method. (*A*) The transmembrane current can be measured as function of externally applied membrane potential. (*B*) Membrane current plotted against membrane potential in absence (I_{tot}) and in presence (I_{ou}) of oubain, an inhibitor of the Na,K pump. (*C*) Ouabain-sensitive current component $\Delta I = I_{tot} - I_{ou}$ generated by the Na,K pump as a function of membrane potential. (Data adapted from Rakowski & Paxson, 1988)

both currents with and without ouabain (Fig. 3*B*) is defined as the Na,K-pump current (Fig. 3*C*).

The advantage of this method is the opportunity to investigate the ion pump in its native environment and in an extended voltage range. No detergent treatment has to be performed and both aqueous compartments can be controlled. The disadvantage is the necessity of discriminating between unspecific currents I_{ou} and the (comparatively small) pump current $\Delta I = I_{tot} - I_{ou}$.

This method was successfully applied to isolated heart cells (Gadsby et al., 1985; Nakao & Gadsby, 1986; Bashinsky, Gadsby & Nakao, 1988), *Xenopus* oocytes (Lafaire & Schwarz, 1986; Rakowski, 1987; Rakowski & Paxson, 1988; Schweigert, Lafaire & Schwarz, 1988) and Na,K pump from *Torpedo* expressed in *Xenopus* oocytes (Schwarz & Gu, 1988).

An unresolved discrepancy exists between the findings of Schwarz and coworkers (Lafaire & Schwarz, 1986; Schweigert et al., 1988; Schwarz & Gu, 1988) and Rakowski and coworker (Rakowski, 1987; Rakowski & Paxson, 1988). Schwarz and coworkers observe a negative slope of the current-voltage function in the voltage range from +20 to +60 mV (and they find significant seasonal variations). Rakowski and Paxson find a monotonous current-voltage function with a slightly positive slope in the range between 0 and 60 mV.

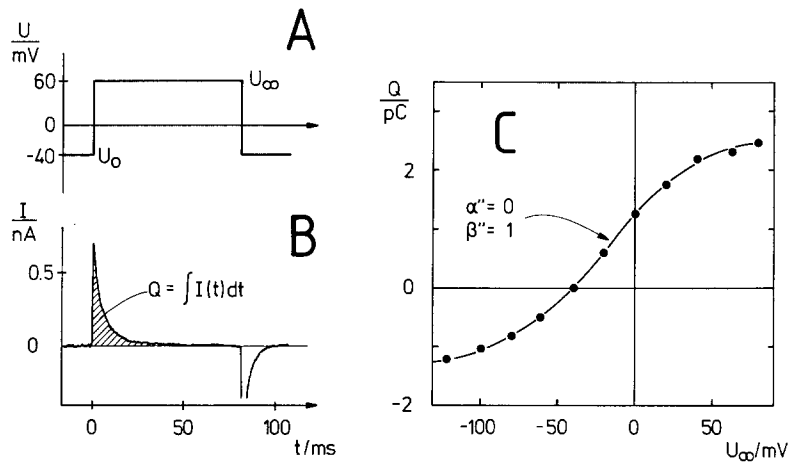

Fig. 4. Current relaxation experiment from cardiac cells under K^+-free conditions (adapted from Nakao & Gadsby, 1986). (*A*) Externally applied voltage jump. (*B*) Ouabain-sensitive, transient current. The hatched area corresponds to the displaced charge while the pump relaxes into a new steady state. (*C*) Voltage-dependent charge displacements. The jumps were performed relative to a holding potential of −40 mV. The line is a calculation according to Läuger and Apell (1988*a*) with the dielectric coefficients $\alpha'' = 0$ and $\beta'' = 1$

With the same setup, Nakao and Gadsby (1986) performed voltage-jump current-relaxation experiments. They determined the oubain-sensitive transient pump current elicited by a sudden displacement of transmembrane voltage in the presence of intracellular ATP and Na^+ but absence of K^+ at both membrane interfaces. An experiment is shown schematically in Fig. 4. When the current transient I_p upon a step-like voltage change is integrated (hatched region in Fig. 4*B*) the charge displacement Q by the pumps perpendicular to the membrane plane is obtained and Q can be plotted against the size of the voltage U_∞. Additional information can be determined from the relaxation time of the current transient.

Na,K Pump Reconstituted in Lipid Vesicles

Since Jørgensen in 1974 published methods to isolate functional preparations of the Na,K pump, several prescriptions of reconstitution of the protein into vesicles were published (Anner et al., 1977; Anner & Moosmayer, 1981; Karlish & Pick, 1981; Karlish & Stein, 1982*a*; Anner, Marcus & Moosmayer, 1984; Apell et al., 1985; Alpes et al., 1988). Different approaches were used to investigate the influence of membrane potential on the pump.

Fluorescein-labeled Na,K pump, which shows a fluorescence quenching upon the transition $E_1P(Na_3) \rightarrow E_2P$, can be examined by stopped-flow techniques (Rephaeli, Richards & Karlish, 1986*a,b*). Membrane potentials are maintained by potassium concentration gradients and the potassium ionophore valinomycin (Hoffman & Laris, 1974) or lithium concentration gradients and the lithium ionophore AS701 (Shanzer, Samuel & Korenstein, 1983).

A second method is the detection of ^{22}Na uptake in proteoliposomes at different membrane potentials (Goldschleger et al., 1987; Karlish et al., 1988). Again membrane potentials were maintained by ion gradients and ionophores. The membrane voltage is traced by a potential-sensitive fluorescence dye DiS-C_3-(5) (Goldschleger et al., 1987).

A third method is the direct use of potential-sensitive fluorescence dyes (Apell et al., 1985; Cornelius & Skou, 1985; Apell & Bersch, 1987, 1988). A typical experiment is described in Fig. 5*A*. Inside-out oriented Na,K pump is activated by addition of ATP and moves Na^+ into and K^+ out of the vesicle, causing an intravesicular positive potential (corresponding to a cytoplasmic negative potential). Right-side-out oriented pumps are not active since ATP does not permeate through the membrane. The fluorescence dye, e.g. oxonol VI, indicates the increase of membrane potential by increase of fluorescence, as shown in Fig. 5*B*. With the known fluorescence-voltage calibration curve (Apell & Bersch, 1987) the current-voltage curve of the Na,K pump can be determined from magnitude and slope of the fluorescence signal (Apell & Bersch, 1988).

The advantage of experiments with vesicles consists in the fact that only the protein of interest, the Na,K pump, is investigated and that experiments are highly reproducible. The change of the intravesicular solution is possible only by dialysis or by freeze-thaw method. The small internal volume provides only a restricted amount of "extracellular" compounds. A disadvantage is the necessary treatment with detergents, which may alter with protein properties.

Purified Microsomal Membrane Sheets Bound to Lipid Bilayers

Nonstationary pump currents after an ATP-concentration jump can be recorded in a compound membrane system (Fig. 6*A*) consisting of flat Na,K-

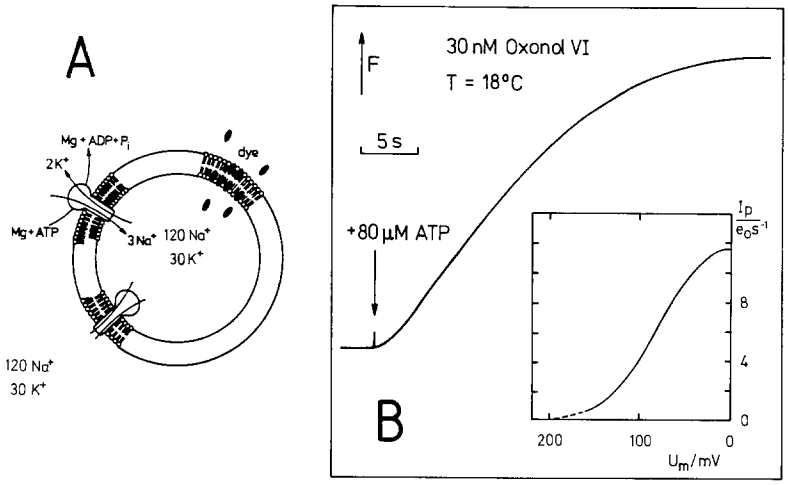

Fig. 5. Na,K pumps generate a transmembrane potential upon addition of ATP to reconstituted vesicles. (*A*) Vesicle structure and enzyme reaction. Ion concentration (120 mM Na^+ and 30 mM K^+) given in the beginning of the experiment is shown. (*B*) Change of fluorescence amplitude upon addition of ATP. An increase of fluorescence refers to an inside more positive potential (Apell & Bersch, 1987). *Inset:* Current-voltage curve determined from fluorescence signal according to Apell and Bersch (1988)

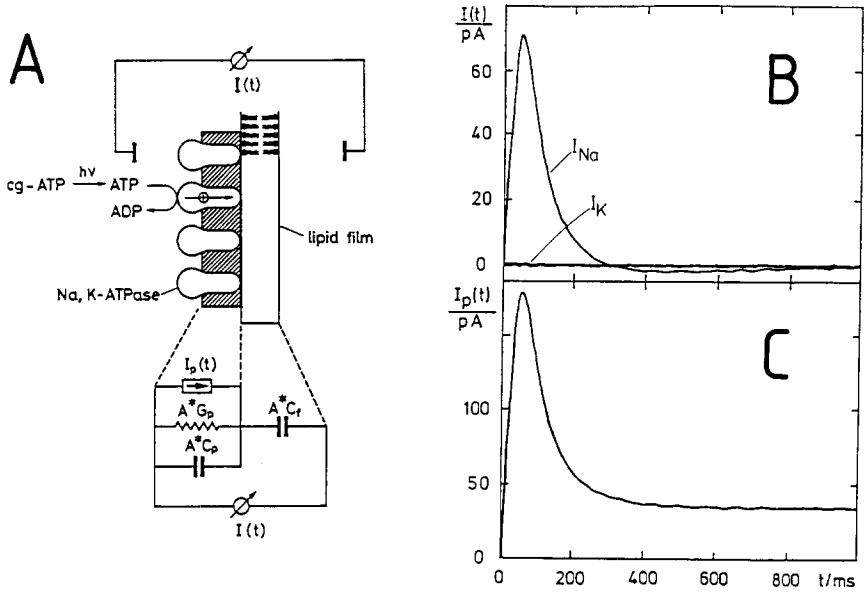

Fig. 6. (*A*) Compound membrane system consisting of a "black" lipid film with adsorbed Na,K-ATPase membrane fragments. Photochemical release of ATP leads to a transient pump current $I_p(t)$. In the external measuring circuit a time-dependent current $I(t)$ is recorded. G_p and C_p are the specific conductance and the specific capacitance of the membrane fragments (referred to unit area); C_f is the specific capacitance of the covered part of the black film. (*B*) Experimental current transient triggered by release of ATP from "caged" ATP in buffers containing 1 mM K_2SO_4 (I_K) and 10 mM Na_2SO_4 (I_{Na}). (*C*) Intrinsic pump current I_p calculated from I_{Na} by a procedure according to Borlinghaus et al. (1987)

ATPase membrane fragments bound to a planar lipid bilayer (Fendler et al., 1985; Borlinghaus, Apell & Läuger, 1987; Apell et al., 1987; Fendler, Grell & Bamberg, 1987; Borlinghaus & Apell, 1988; Nagel et al., 1988; Stürmer et al., 1989). The membrane fragments that can be isolated from kidney medulla contain Na,K-ATPase at a high density (several thousand oriented pump molecules per μm^2). When added to the aqueous solution adjacent to the lipid bilayer they bind to the bilayer. By an intense light-flash ATP is released in the solution within a few milliseconds from an ATP derivative with a photolabile blocking group ("caged" ATP). The released ATP activates those membrane fragments attached to the planar bilayer with the ATP binding site facing the solution (Fig. 6*A*).

In the presence of sodium and in the absence of potassium a transient current signal is observed after the ATP concentration jump, corresponding to a translocation of positive charge in the protein layer towards the lipid film (Fig. 6*B*). When sodium is replaced by potassium or when inhibitors of the Na,K-ATPase, such as ouabain or vanadate, are added to the solution, the current signal is abolished (Fig. 6*B*). From the observed current signal $I(t)$ the intrinsic pump current $I_p(t)$ can be evaluated using estimated values of the circuit parameters G_p, C_p and C_f (Borlinghaus et al., 1987) as shown in Fig. 6*C*.

The advantage of this method is the direct access to current or voltage signals of electrogenic actions of the Na,K pump. The composition of the "cytoplasmic" solution can be controlled easily. The disadvantage of this method is the almost undefined state of the "extracellular" interface of the membrane sheets, which is the narrow cleft be-

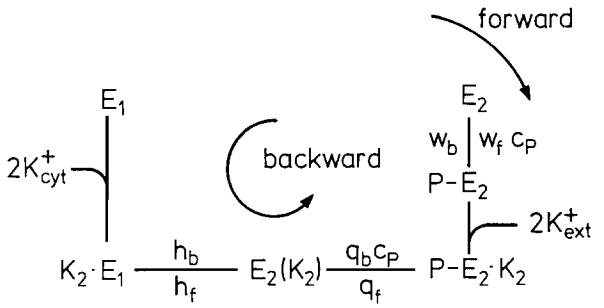

Fig. 7. Reaction sequence of the Na,K pump under conditions of K-K exchange. h_f, q_f, $w_f c_P$ and h_b, $q_b c_P$, w_b are rate constants for transitions in "forward" and "backward" direction, respectively. c_P is the cytoplasmic concentration of inorganic phosphate

tween membrane fragments and supporting lipid bilayer (Fig. 6*A*). Although it has been shown that a limited amount of potassium is present when the bulk solution contains potassium, but the (nondeterminable) concentration in the cleft reaches a steady state only within minutes (Borlinghaus et al., 1987; Borlinghaus, 1988). To overcome this difficulty, experiments were performed with ionophore and uncoupler doped supporting lipid bilayers (Fendler et al., 1985; Nagel et al., 1988).

The Interpretation of Various Experiments Allows the Analysis of Partial Reactions

As shown in the introduction, the description of electrogenic actions of the Na,K pump needs not only the rate constants of the partial reactions but also dielectric coefficients and the charge of the ion binding sites. In the following, results are compiled from different approaches to determine (at least qualitatively) values for the relevant parameters.

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 K^+-K^+ exchange (Glynn, Lew & Lüthi, 1970; Glynn, Hoffman & Lew, 1971). A simplified reaction scheme (Karlish & Yates, 1978; Sachs, 1981; Karlish & Stein, 1982*a*,*b*) that 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 (Post, Toda & Rogers, 1975; Schuurmans Stekhoven et al., 1980) and that binding of K^+ to $P - E_2$ results in dephosphorylation and formation of the occluded state $E_2(K_2)$ (Blostein & Chu, 1977; Glynn & Richards, 1982). ATP is known to bind to the occluded state and to increase the rate of the transition $E_2(K_2) \rightarrow K_2 \cdot E_1$ (Glynn, 1985). The same effect is observed with nonphosphorylating analogs of ATP (Simons, 1975), 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. A theoretical treatment of the K^+-K^+ exchange is given by Läuger and Apell (1988*b*). The rate constants can be expressed in analogy to k_f and k_b (Eq. (7))

$$h_f = \bar{h}_f \exp\{(2 + z_L)\gamma' u/2\}. \tag{18}$$

The rate constant h_b of the backward transition is obtained by changing the sign of the exponent.

Using reconstituted lipid vesicles Goldschleger et al. (1987) have studied the (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). Further evidence for the electroneutrality of the K^+-K^+ exchange is given by Bashinski et al. (1988), who investigated guinea pig ventricular myocytes. They found no electrogenic phenomena in sodium-free potassium solutions. Recently published experiments on *Xenopus* oocytes exhibit also a membrane-potential independent pump-mediated current in sodium-free medium (Rakowski & Paxson, 1988; Rakowski et al., 1989). Data of experiments performed by Stürmer et al. (1989) with purified microsomal membranes bound to lipid bilayers proved that electrogenic effects in sodium-free potassium solutions (1–10 mM) are less than 1% of that obtained after the addition of 10 mM Na^+. All these experimental findings are consistent with the assumption that the transitions $K_2 \cdot E_1 \rightleftarrows E_2(K_2)$ and $E_2(K_2) \rightleftarrows E_2 \cdot K_2$ are electroneutral.

First conclusions to be drawn from these results are that q_f, q_b, h_f and h_b are voltage independent, which means that z_L must be −2 and/or $\gamma' = \gamma'' = 0$. On the other hand, it does not exclude the possibility that binding and dissociation of K^+ at the extracellular site are electrogenic (corresponding to the presence of a "potassium well"), since the experiments of Bashinski et al. (1988) have been carried out at saturating extracellular potassium concentration (c''_K = 5.4 mM). At high c''_K, the extracellular sites are predominantly in the potassium-loaded form ($E_2 \cdot K_2$), so that a voltage change is unable to shift the equilibrium between E_2 and $E_2 \cdot K_2$ appreciably, even if the equilibrium constant is voltage dependent. However, a transient current should be observed at intermediate potassium concentrations

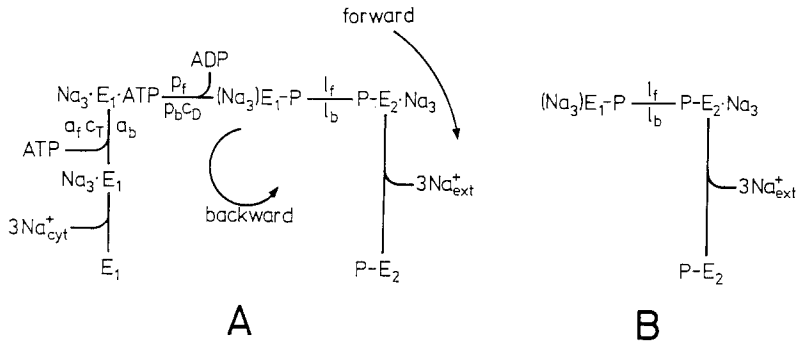

Fig. 8. (*A*) Transport reactions of the Na,K pump in the absence of potassium based on the Albers-Post scheme of the pumping cycle. Nomenclature as in Fig. 1. a_f and a_b are the rate constants of ATP binding and dissociation in the absence of potassium. (*B*) Remaining transport reactions of the Na,K pump in the absence of potassium and ADP and in the presence of ATP

($c''_K \approx K''_K$), if an extracellular ion well exists. The same arguments are valid for the data of Goldschleger et al. (1987) because these experiments have been done at saturating extracellular (intravesicular) Rb^+ concentrations (140–285 mM). But when 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 ($\alpha' \approx 0$). Stürmer et al. (1989) draw the same conclusions from their data.

Sodium-Only Mode

The Na,K pump can perform different modes of sodium transport in the absence of potassium, depending on intra- and extracellular sodium concentrations and on the presence of intracellular ATP and ADP (for review, *see* Glynn, 1985). In the following, "sodium-only mode" refers to all reactions in the absence of potassium.

Under potassium-free conditions and in absence of ADP a transport cycle of the pump can be derived from the Albers-Post scheme as shown in Fig. 8*A* (Läuger & Apell, 1988*a*,*b*). The transition from $P - E_2$ to E_1 involving dephosphorylation of the enzyme is extremely slow (Post et al., 1972; Karlish & Yates, 1978; Schuurmanns Stekhoven et al., 1986). Besides the physiological mode of pumping, the sodium-only mode was also found to be electrogenic and to exhibit a stoichiometry of 3 Na^+ transferred per ATP hydrolysed (Glynn & Karlish, 1976; Cornelius & Skou, 1985; Fendler et al., 1985; Borlinghaus et al., 1987; Karlish et al., 1988). Since the access of ions from the cytoplasmic phase is electrically silent ($\alpha' = 0$, *see* above), the electrogenic effect has to occur with the transitions from Na_3E_1ATP to $E_2 - P$.

Borlinghaus et al. (1987) observed that treatment of microsomal membrane fragments with α-chymotrypsin abolishes the transient current, which is normally seen after the ATP-concentration jump. Treatment of Na,K pump with α-chymotrypsin in the presence of sodium leads to a cleavage of a single peptide bond in the α-subunit between Leu-266 and Ala-267 (Jørgensen & Collins, 1986). Enzyme modified in this way can be phosphorylated by ATP, can occlude 3 Na^+, and ATP/ADP exchange is possible while Na^+ pumping or transmembrane Na^+-Na^+ exchange is disabled (Glynn, Hara & Richards, 1984; Jørgensen & Petersen, 1985). These findings indicate that phosphorylation by ATP and occlusion of sodium are electrically silent steps (i.e., $\beta' = 0$). Apell et al. (1987) have shown that the evidence of experiments done by Borlinghaus et al. (1987) is not sufficient to discriminate as the source of electrogenic effects between deocclusion of sodium ions (β'') and the release of sodium ions (α'').

Nakao and Gadsby (1986) recently described experiments with cardiac cells in which transient pump currents were elicited by a sudden displacement of transmembrane voltage. The ouabain-sensitive component of the current was identified with the current I_p generated by the Na,K pump. The transient pump current was observed in the absence of extracellular potassium, but required the presence of intracellular ATP and both the presence of intra- and extracellular Na^+. A voltage jump from a holding potential of −40 to +60 mV resulted in an outward-directed transient current which decayed nearly exponentially to zero with a time constant of about 5 msec. Voltage jumps to more negative potentials gave rise to transient inward currents.

Under the conditions of the experiments of Nakao and Gadsby, i.e., in the (nominal) absence of ADP, the reaction $Na_3 \cdot E_1 \cdot ATP \rightleftarrows (Na_3)E_1 - P$ (Fig. 8*A*) is shifted far to the right. Furthermore, since the pump current after the voltage jump decayed to virtually zero, one may infer that the rate of the transition $P - E_2 \rightarrow E_1$ was negligibly small. Under these circumstances the reaction scheme of Fig. 8*A* reduces to the deocclusion of sodium ions and of the release of sodium ions (*see* Fig. 8*B*). This means that under the given experimental conditions

(absence of extracellular K^+ and of intracellular ADP), a quasi-equilibrium exists between states $(Na_3)E_1 - P$, $P - E_2 \cdot Na_3$, $P - E_2 \cdot Na_2$, $P - E_2 \cdot Na$ and $P - E_2$ of the pump.

A voltage jump leads to a shift of this quasi-equilibrium and to a concomitant transient current $I_p(t)$, depending on the magnitude of the dielectric coefficients α'' and β'' (Läuger & Apell, 1988*a*). The voltage dependence of the transferred charge, which is the time integral of the transient $I_p(t)$, can be described completely only by adjusting $\alpha'' = 0$ and $\beta'' = 1$. The analysis of the voltage dependence of the relaxation time of the transient current at different voltage jumps leads to a different set of dielectric parameters ($\alpha'' = 0.5$ and $\beta'' = 0.3$). The discrepancy between both results cannot be resolved so far. It is noteworthy that the analysis of relaxation time also implies a electrogenic contribution of α' and/or β', which can be excluded as shown above. Although the magnitude of β'' is not determined uniquely so far, it can be stated that it is significantly greater than zero. Consequently, from Eq. (5) it can be seen that z_L must be greater than -3 to account for the observed electrogenic effect.

Net Charge of the Ion-Binding Sites

Nakao and Gadsby (1986) determined from the quantity of charge moved in their relaxation experiments that one positive charge is translocated across the membrane. This corresponds to a charge of the binding sites $z_L = -2$.

In the light of the finding that $\beta' + \beta'' \neq 0$ the condition $\gamma' + \gamma'' \neq 0$ has to be fulfilled, and therefore the electroneutrality of K^+-K^+ exchange can be understood only under the assumption that $z_L = -2$ (*see above*). Goldschleger et al. (1987) also proposed from the voltage independence of Rb^+-Rb^+ exchange that the ligand system bears a charge of $z_L = -2$.

Läuger and Apell (1986) showed that this conclusion is consistent with the observed monotonic shape of the current-voltage characteristic of the Na,K pump in cardiac cells (Gadsby et al., 1985), squid giant axon (Rakowski & Paxson, 1988) and reconstituted vesicles (Apell & Bersch, 1988).

In addition, the condition $z_L = -2$ supports the molecular interpretation of a first-order Michaelis-Menten type dependence of the Na,K pump on the cytoplasmic sodium concentration (Karlish & Stein, 1985; Apell & Marcus, 1986; Borlinghaus & Apell, 1988). They suggested two negatively charged and one neutral sodium-binding sites. In this case, the binding sites are not equivalent, and for electrostatic reasons the affinity of sodium at the neutral site could be considerably reduced. Due to the characteristics of the third sodium ion-binding site the concentration of the state $Na_3 \cdot E_1 \cdot ATP$ should become limiting at low cytoplasmic sodium.

Conclusion

An analysis of the Na,K pump can be performed on the base of the Albers-Post reaction scheme in which the overall transport process is described as a sequence of conformational transitions and ion-binding and ion-release steps. It reveals that the electrogenicity of the transport processes can be determined by a set of dielectric coefficients reflecting the magnitude of charge translocations associated with individual reaction steps and by the net charge of the ion-binding sites.

By putting together the available experimental evidence, a consistent description of the Na,K pump can be obtained as follows:

a) the binding sites bear two negative charges ($z_L = -2$), and therefore the transitions $K_2 \cdot E_1 \rightleftarrows E_2(K_2)$ and $E_2(K_2) \rightleftarrows E_2 \cdot K_2$ are electroneutral;

b) the dielectric distance between cytoplasm and cytoplasmic binding sites is negligible ($\alpha' \approx 0$), which could be explained by a wide waterfilled access channel;

c) the phosphorylation by ATP and occlusion of sodium are electrically silent steps ($\beta' = 0$);

d) the deocclusion of sodium ions and the release of sodium ions on the extracellular interface are the only candidates for charge-translocating steps ($\beta'' + \alpha'' \approx 1$);

e) although it has been shown that the deocclusion contributes significantly to the electrogenic effect ($\beta'' > 0$), up to now the evidence is sufficient to claim that it is the sole charge-translocating step (i.e., $\beta'' \approx 1$).

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References

Albers, R.W. 1967. *Annu. Rev. Biochem.* **36:**727–756

Alpes, H., Apell, H.-J., Knoll, G., Plattner, H., Riek, R. 1988. *Biochim. Biophys. Acta* **946:**379–388

Anner, B.M., Lane, L.K., Schwartz, A., Pitts, B.J.R. 1977. *Biochim. Biophys. Acta* **467:**340–345

Anner, B.M., Marcus, M.M., Moosmayer, M. 1984. *In:* Enzymes, Receptors and Carriers of Biomembranes. A. Azzi et al., editors. pp. 81–96. Springer-Verlag, Heidelberg

Anner, B.M., Moosmayer, M. 1981. *J. Biochem. Biophys. Meth.* **5:**299–306

Apell, H.-J., Bersch, B. 1987. *Biochim. Biophys. Acta* **903:**480–494

Apell, H.-J., Bersch, B. 1988. *In:* The Na^+,K^+-Pump. Part A: Molecular Aspects. J.C. Skou et al., editors. pp. 421–434. A.R. Liss, New York

Apell, H.-J., Borlinghaus, R., Läuger, P. 1987. *J. Membrane Biol.* **97:**179–191

Apell, H.-J., Marcus, M.M. 1986. *Biochim. Biophys. Acta* **862:**254–264

Apell, H.-J., Marcus, M.M., Anner, B.M., Oetliker, H., Läuger, P. 1985. *J. Membrane Biol.* **85:**49–63

Bashinski, A., Gadsby, D.C., Nakao, M. 1988. *Proc. Natl. Acad. Sci. USA* **85:**3412–3416

Beaugé, L.A., Sjodin, R.A. 1976. *J. Physiol. (London)* **263:**383–403

Blaustein, M.P., Lieberman, M. (editor). 1984. Society of General Physiologists Series, Vol. 38. Raven, New York

Blostein, R., Chu, L. 1977. *J. Biol. Chem.* **252:**3035–3043

Borlinghaus, R. 1988. PhD. Thesis, University of Konstanz, Konstanz

Borlinghaus, R., Apell, H.-J. 1988. *Biochim. Biophys. Acta* **939:**197–206

Borlinghaus, R., Apell, H.-J., Läuger, P. 1987. *J. Membrane Biol.* **97:**161–178

Cantley, L.C. 1981. *Curr. Top. Bioenerg.* **11:**201–237

Cantley, L.C., Carili, C.T., Smith, R.L., Perlman, D. 1984. *Curr. Top. Membr. Transp.* **19:**315–322

Cornelius, F., Skou, J.C. 1985. *Biochim. Biophys. Acta* **818:**211–221

DeWeer, P., Gadsby, D.C., Rakowski, R.F. 1986. *J. Physiol. (London)* **371:**144P

DeWeer, P., Gadsby, D.C., Rakowski, R.F. 1988*a*. *In:* The Na^+,K^+-Pump. Part A: Molecular Aspects. J.C. Skou et al., editors. pp. 469–476. A.R. Liss, New York

DeWeer, P., Rakowski, R.F., Gadsby, D.C. 1988*b*. *Annu. Rev. Physiol.* **50:**225–241

Eisner, D.A., Lederer, W.J. 1980. *J. Physiol. (London)* **303:**441–474

Fendler, K., Grell, E., Bamberg, E. 1987. *FEBS Lett.* **244:**83–88

Fendler, K., Grell, E., Haubs, M., Bamberg, E. 1985. *EMBO J.* **4:**3079–3085

Gadsby, D.C. 1984. *Annu. Rev. Biophys. Bioeng.* **13:**373–398

Gadsby, D.C., Kimura, J., Noma, A. 1985. *Nature (London)* **315:**63–65

Gadsby, D.C., Rakowski, R.F., DeWeer, P. 1986. *Biophys. J.* **49:**36a

Glasstone, S., Laidler, K.J., Eyring, H. 1941. The Theory of Rate Processes. McGraw-Hill, New York

Glitsch, H.G. 1982. *Annu. Rev. Physiol.* **44:**389–400

Glynn, I.M. 1985. *In:* The Enzymes of Biological Membranes. (2nd ed.) Membrane Transport. Vol. 3, pp. 35–114. A.N. Martonosi, editor. Plenum, New York

Glynn, I.M., Hara, Y., Richards, D.E. 1984. *J. Physiol. (London)* **256:**465–496

Glynn, I.M., Hoffman, J.F., Lew, V.L. 1971. *Phil. Trans. R. Soc. London B* **262:**81–102

Glynn, I.M., Karlish, S.J.D. 1976. *J. Physiol. (London)* **256:**465–496

Glynn, I.M., Lew, V.L., Lüthi, V. 1970. *J. Physiol. (London)* **207:**371–391

Glynn, I.M., Richards, D.E. 1982. *J. Physiol. (London)* **330:**17–43

Goldschleger, R., Karlish, S.J.D., Rephaeli, A., Stein, W.D. 1987. *J. Physiol. (London)* **387:**331–355

Hamill, O.P., Marty, A., Neher, E., Sakmann, B., Sigworth, F.J. 1981. *Pflüegers Arch.* **391:**85–100

Hodgin, A.L., Keynes, R.D. 1955. *J. Physiol. (London)* **128:**28–60

Hoffman, J.F., Laris, P.C. 1974. *J. Physiol. (London)* **239:**519–552

Jauch, P., Petersen, O.H., Läuger, P. 1986. *J. Membrane Biol.* **94:**99–115

Jørgensen, P.L. 1974. *Biochim. Biophys. Acta* **356:**36–52

Jørgensen, P.L. 1982. *Biochim. Biophys. Acta* **694:**27–68

Jørgensen, P.L., Andersen, J.P. 1988. *J. Membrane Biol.* **103:**95–120

Jørgensen, P.L., Collins, J.H. 1986. *Biochim. Biophys. Acta* **860:**570–576

Jørgensen, P.L., Petersen, J. 1985. *Biochim. Biophys. Acta* **821:**319–333

Kaplan, J.H. 1985. *Annu. Rev. Physiol.* **47:**535–544

Karlish, S.J.D., Goldshleger, R., Shahak, Y., Rephaeli, A. 1988. *In:* The Na^+,K^+-Pump. Part A: Molecular Aspects. J.C. Skou et al., editors. pp. 519–524. A.R. Liss, New York

Karlish, S.J.D., Pick, U. 1981. *J. Physiol. (London)* **312:**505–529

Karlish, S.J.D., Stein, W.D. 1982*a*. *J. Physiol. (London)* **328:**295–316

Karlish, S.J.D., Stein, W.D. 1982*b*. *J. Physiol. (London)* **328:**317–331

Karlish, S.J.D., Stein, W.D. 1985. *J. Physiol. (London)* **359:**119–149

Karlish, S.J.D., Yates, D.W. 1978. *Biochim. Biophys. Acta* **527:**115–130

Lafaire, A.V., Schwarz, W. 1986. *J. Membrane Biol.* **91:**43–51

Läuger, P. 1984. *Biochim. Biophys. Acta* **779:**307–341

Läuger, P., Apell, H.-J. 1986. *Eur. Biophys. J.* **13:**309–321

Läuger, P., Apell, H.-J. 1988*a*. *Biochim. Biophys. Acta* **944:**451–464

Läuger, P., Apell, H.-J. 1988*b*. *Biochim. Biophys. Acta* **945:**1–10

Läuger, P., Apell, H.-J. 1989. *In:* Monovalent Cations in Biological Systems. C.A. Pasternak, editor. *CRC Crit. Rev. (in press)*

Läuger, P., Benz, R., Stark, G., Bamberg, E., Jordan, P.C., Fahr, A., Brock, W. 1981. *Q. Rev. Biophys.* **14:**513–598

Lederer, W.J., Nelson, M.T. 1984. *J. Physiol. (London)* **348:**665–677

Milanick, M.A., Hoffman, J.F. 1986. *Biophys. J.* **49:**548a

Mitchell, P., Moyle, J. 1974. *Biochem. Soc.* (Spec. Publ.) **4:**91–111

Nagel, G., Fendler, K., Grell, E., Bamberg, E. 1988. *Biochim. Biophys. Acta* **901:**239–249

Nakao, M., Gadsby, D.C. 1986. *Nature (London)* **323:**628–630

Parlin, R.B., Eyring, H. 1954. *In:* Ion Transport across Membranes. H.T. Clarke, editor. pp. 103–118. Academic, New York

Post, R.L., Hegyvary, C., Kume, S. 1972. *J. Biol. Chem.* **247:**6530–6540

Post, R.L., Toda, G., Rogers, F. 1975. *J. Biol. Chem.* **250:**691–701

Rakowski, R.F. 1987. *J. Gen. Physiol.* **90:**34a

Rakowski, R.F., Gadsby, D.C., DeWeer, P. 1989. *J. Gen. Physiol.* **93:**903–941

Rakowski, R.F., Paxson, C.L. 1988. *J. Membrane Biol.* **106:**173–182

Rephaeli, A., Richards, D.E., Karlish, S.J.D. 1986*a*. *J. Biol. Chem.* **261:**6248–6254

Rephaeli, A., Richards, D.E., Karlish, S.J.D. 1986*b*. *J. Biol. Chem.* **261:**12437–12440

Robinson, J.D., Flashner, M.S. 1979. *Biochim. Biophys. Acta* **549:**145–176

Sachs, J.R. 1981. *J. Physiol. (London)* **316:**263–277
Schuurmans Stekhoven, F., Bonting, S.L. 1981. *Physiol. Rev.* **61:**1–76
Schuurmans Stekhoven, F.M.A.H., Swarts, H.G.P., DePont, J.J.H.M.M., Bonting, S.L. 1980. *Biochim. Biophys. Acta* **597:**100–111
Schuurmans Stekhoven, F.M.A.H., Swarts, H.G.P., DePont, J.J.H.M.M., Bonting, S.L. 1986. *Biochim. Biophys. Acta* **855:**375–382
Schwarz, W., Gu, Q. 1988. *Biochim. Biophys. Acta* **945:**167–174
Schweigert, B., Lafaire, A.V., Schwarz, W. 1988. *Pfluegers Arch.* **412:**579–588
Shanzer, A., Samuel, D., Korenstein, R. 1983. *J. Am. Chem. Soc.* **105:**3815–3818
Simons, T.J.B. 1975. *J. Physiol. (London)* **244:**731–739
Skou, J.C. 1975. *Q. Rev. Biophys.* **7:**401–431
Slayman, C. (editor). 1982. *Curr. Top. Membr. Transp.* **16**
Soejima, M., Noma, A. 1984. *Pfluegers Arch.* **400:**424–431
Stein, W.D. 1986. Transport and Diffusion across Cell Membranes. Chap. 6. Academic, Orlando
Stürmer, W., Apell, H.-J., Wuddel, I., Läuger, P. 1989. *J. Membrane Biol.* **110:**67–86
Thomas, R.C. 1972. *Physiol. Rev.* **52:**563–594
Warshel, A., Russel, S.T. 1984. *Q. Rev. Biophys.* **17:**283–422

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