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In Memoriam Peter F. Baker

Voltage Dependence of Sodium-Calcium Exchange: Predictions from Kinetic Models

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Summary. Voltage effects on the Na-Ca exchange system are analyzed on the basis of two kinetic models, a "consecutive" and a "simultaneous" reaction scheme. The voltage dependence of a given rate constant is directly related to the amount of charge which is translocated in the corresponding reaction step. Charge translocation may result from movement of an ion along the transport pathway, from displacement of charged ligand groups of the ion-binding site, or from reorientation of polar residues of the protein in the course of a conformational transition. The voltage dependence of ion fluxes is described by a set of coefficients reflecting the dielectric distances over which charge is translocated in the individual reaction steps. Depending on the charge of the ligand system and on the values of the dielectric coefficients, the flux-voltage curve can assume a variety of different shapes. When part of the transmembrane voltage drops between aqueous solution and binding site, the equilibrium constant of ion binding becomes a function of membrane potential. By studying the voltage dependence of ion fluxes in a wide range of sodium and calcium concentrations, detailed information on the microscopic properties of the transport system may be obtained.

Key Words Na-Ca exchange · electrogenic transport · current-voltage characteristic · membrane potential · transport models

Introduction

The plasma membrane of many excitable cells contains a Na-Ca exchange system which mediates countertransport of sodium and calcium. The Na-Ca exchanger has been extensively studied in squid giant axon (Baker et al., 1967), in heart muscle (Reuter & Seitz, 1968), in plasma-membrane vesicles derived from cardiac cells (Pitts, 1979; Reeves & Sutko, 1979) and in reconstituted vesicles (Barzilai, Spanier & Rahaminoff, 1984; Hale et al., 1984); for recent reviews, *see* Reuter, 1982; Langer, 1982; DiPolo & Beaugé, 1983; Requena, 1983; Mullins, 1984; Carafoli, 1985; Philipson, 1985; Baker, 1986; Eisner & Valdeolmillos, 1986. Depending on the magnitude of the electrochemical gradients, the Na-Ca exchange system is engaged in calcium extrusion coupled to sodium entry or in calcium entry coupled to sodium extrusion. Numerous studies have shown that the countertransport of sodium and calcium is voltage dependent and is associated with the transfer of net charge across the membrane (Mullins & Brinley, 1975; Sjodin & Abercrombie, 1978; Mullins, 1979; Caroni, Reinlieb & Carafoli, 1980; Philipson & Nishimoto, 1980; Ledvora & Hegyvary, 1983; Nelson & Lederer, 1984; Reeves & Hale, 1984; DiPolo et al., 1985; Eisner & Lederer, 1985; Kimura, Noma & Irisawa, 1986; Allen & Baker, 1986*a*,*b*; Kimura, Miyamae & Noma, 1987). Determinations of the reversal potential (Reeves & Hale, 1984) and of the isotope flux ratio (Baker et al., 1969; Pitts, 1979), as well as studies of the sodium-concentration dependence of calcium fluxes (Blaustein, 1977) indicate that the stoichiometry of transport is 3 Na : 1 Ca.

The electrogenic nature of the Na-Ca exchange system has interesting implications. The flux depends on an additional parameter, the membrane voltage. Under nonstationary conditions, such as during the action potential in cardiac cells, the transport system may contribute to the transmembrane current (Mullins, 1979). Furthermore, by studying the fluxes of Na^+ and Ca^{2+} as functions of membrane potential, information on the transport mechanism may be obtained (Hansen et al., 1981; Eisner & Lederer, 1985; Johnson & Kootsey, 1985). In the following, we analyze the voltage dependence of sodium and calcium fluxes on the basis of microscopic models of the transport system.

Two different mechanisms for Na-Ca exchange, usually termed "consecutive" and "simultaneous" have been discussed in the literature. In the simultaneous model (Mullins, 1977; Edmonds, 1986) the exchanger E binds sodium and calcium, forming a complex Na_3ECa; translocation of Na^+ and Ca^{2+} occurs in a single reaction step which may consist in

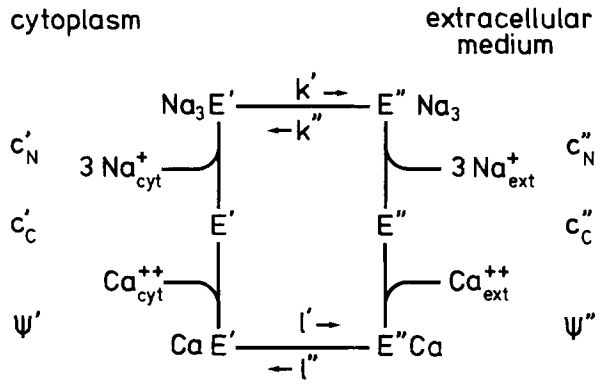

Fig. 1. Consecutive mechanism for countertransport of sodium and calcium. The exchanger E undergoes transitions between a conformation E' (ion binding sites facing the cytoplasm) and a conformation E'' (ion binding sites facing the extracellular medium). c'_N and c'_C are the concentrations of Na^+ and Ca^{2+} in the cytoplasm; c''_N and c''_C are the concentrations in the extracellular medium. ψ' and ψ'' are the electric potentials. The rate constants for conformational transitions in the sodium-loaded state and in the calcium-loaded state are denoted by k', k'', l' and l''

a conformational transition $Na_3E'Ca \rightarrow CaE''Na_3$ of the transport protein. In the consecutive (or "ping-pong") model (Wong & Bassingthwaighte, 1981; Eisner & Lederer, 1985), translocation of sodium and calcium are separate events. So far, the experimental evidence is not sufficient for an unambiguous discrimination between simultaneous and consecutive reaction models (Blaustein, 1977; DiPolo, 1979; Ledvora & Hegyvary, 1983; Johnson & Kootsey, 1985). In the following we discuss for both models the dependence of ion fluxes on concentrations and voltage. Numerical examples for the flux-voltage relation will be given only for the consecutive mechanism. This preference derives from the potentially greater simplicity of the consecutive model, requiring only one kind of ligand system which binds either calcium with high affinity or sodium with low affinity. Experimental criteria for the distinction between simultaneous and consecutive models will be discussed in a later section.

Consecutive Mechanism for Na-Ca Exchange

Steady-State Fluxes

The analysis of the consecutive transport model is based on the reaction scheme of Fig. 1 in which it is assumed that the exchanger E undergoes transitions between a conformation E' with ion-binding sites facing the cytoplasm and a conformation E'' with ion-binding sites facing the extracellular medium. For the evaluation of the steady-state fluxes, the following additional assumptions are introduced:

a) the exchanger can be present only in states E, $E\mathrm{Na}$, $E\mathrm{Na}_2$, $E\mathrm{Na}_3$ and $E\mathrm{Ca}$; mixed complexes such as $E\mathrm{CaNa}$ are neglected. Furthermore, the three sodium binding sites are considered to be equivalent.

b) Transitions between conformations E' and E'' are possible only in states $E\mathrm{Na}_3$ and $E\mathrm{Ca}$; this means that the net fluxes of Na^+ and Ca^{2+} are completely coupled.

c) The rate constants of ion binding and release are much larger than the rate constants of conformational transitions so that the binding and release reactions are always in equilibrium. This assumption is consistent with the observation (Eigen & Maass, 1966) that rate constants for the association of alkali ions with ligands are close to the limit of diffusion-controlled reactions ($k_a \approx 10^9$ M^{-1} sec^{-1}). The existence of a binding equilibrium is less obvious for Ca^{2+}, which also exhibits high association rates (Eigen & Maass, 1966), but binds more strongly than Na^+. The equilibrium assumption is introduced here merely for simplicity; it may be replaced by a more refined treatment as more experimental information becomes available.

Denoting the fraction of exchanger molecules which are in state A by $x[A]$, the binding reactions at the cytoplasmic site are described by equilibrium constants K'_{N1}, K'_{N2}, K'_{N3} and K'_C:

$$K'_{N1} = \frac{x[E']}{x[\mathrm{Na}E']}\, c'_N; \quad K'_{N2} = \frac{x[\mathrm{Na}E']}{x[\mathrm{Na}_2E']}\, c'_N \tag{1}$$

$$K'_{N3} = \frac{x[\mathrm{Na}_2E']}{x[\mathrm{Na}_3E']}\, c'_N; \quad K'_C = \frac{x[E']}{x[\mathrm{Ca}E']}\, c'_C. \tag{2}$$

c'_N and c'_C are the cytoplasmic concentrations of Na^+ and Ca^{2+}, respectively. Analogous equations hold for the extracellular site. The equilibrium constants as well as the translocation rate constants k', k'', l' and l'' (Fig. 1) depend, in general, on membrane potential $V = \psi' - \psi''$, as will be discussed in more detail later. According to the principle of microscopic reversibility, the kinetic constants are connected by

$$\frac{k'l''}{k''l'} \cdot \frac{K''_{N1}K''_{N2}K''_{N3}}{K'_{N1}K'_{N2}K'_{N3}} \cdot \frac{K'_C}{K''_C} = \exp(u) \tag{3}$$

$$u \equiv \frac{\psi' - \psi''}{kT/e_o} = \frac{V}{kT/e_o}. \tag{4}$$

k is the Boltzmann constant, T the absolute temperature and e_o the elementary charge. For a derivation of Eq. (3), *see* Läuger (1984).

Quantities of experimental interest are the unidirectional outward fluxes Φ'_N and Φ'_C of sodium and

calcium, the unidirectional inward fluxes Φ''_N and Φ''_C, and the net (outward) fluxes Φ_N and Φ_C which are given by

$$\Phi_N \equiv \Phi'_N - \Phi''_N; \qquad \Phi_C \equiv \Phi'_C - \Phi''_C. \tag{5}$$

According to the assumption of interfacial equilibrium, the unidirectional fluxes (which are referred to a single exchanger molecule) are given by

$$\Phi'_N = 3k'x[\mathrm{Na_3}E']; \qquad \Phi''_N = 3k''x[E''\mathrm{Na_3}] \tag{6}$$

$$\Phi'_C = l'x[\mathrm{Ca}E']; \qquad \Phi''_C = l''x[E''\mathrm{Ca}]. \tag{7}$$

The fluxes can be evaluated in a straightforward way using Eqs. (1)–(3) together with the steady-state condition

$$k'x[\mathrm{Na_3}E'] - k''x[E''\mathrm{Na_3}] = l''x[E''\mathrm{Ca}] - l'x[\mathrm{Ca}E']. \tag{8}$$

The result reads:

$$\Phi'_N = \frac{3k'}{\rho} \cdot \frac{c'^3_N c''_C}{K'_{N1}K'_{N2}K'_{N3}K''_C}\left(l'' + k''\frac{c''^3_N K''_C}{K''_{N1}K''_{N2}K''_{N3}c''_C}\right) \tag{9}$$

$$\Phi'_C = \frac{l'}{\rho} \cdot \frac{c''^3_N c'_C}{K''_{N1}K''_{N2}K''_{N3}K'_C}\left(k'' + l''\frac{K''_{N1}K''_{N2}K''_{N3}c''_C}{K''_C c''^3_N}\right) \tag{10}$$

$$\Phi_C = -\frac{1}{3}\Phi_N \tag{11}$$

$$= \frac{1}{\rho} \cdot \frac{k''l'}{K''_{N1}K''_{N2}K''_{N3}K'_C} \cdot [c''^3_N c'_C - c'^3_N c''_C \exp(u)]$$

$$\rho \equiv P'Q'' + P''Q' \tag{12}$$

$$P' \equiv 1 + \frac{c'_N}{K'_{N1}} + \frac{c'^2_N}{K'_{N1}K'_{N2}} + \frac{c'^3_N}{K'_{N1}K'_{N2}K'_{N3}} + \frac{c'_C}{K'_C} \tag{13}$$

$$Q' \equiv l'\frac{c'_C}{K'_C} + k'\frac{c'^3_N}{K'_{N1}K'_{N2}K'_{N3}}. \tag{14}$$

Φ''_N, Φ''_C, P'' and Q'' are obtained by exchanging the superscripts $'$ and $''$ in Eqs. (9), (10), (13) and (14). (Note that, under the condition of complete coupling, the relations $\Phi''_N = 3\Phi'_C$, $\Phi'_N = 3\Phi''_C$ hold).

Equations (9)–(11) describe Na-Ca exchange as well as Na-Na and Ca-Ca exchange. From Eq. (10) the rate $\Phi'_C = \Phi''_C$ of Ca-Ca exchange in the absence of sodium is given by

$$\frac{1}{\Phi'_C} = \frac{1}{\Phi''_C} = \frac{1}{l'}\left(1 + \frac{K'_C}{c'_C}\right) + \frac{1}{l''}\left(1 + \frac{K''_C}{c''_C}\right) \tag{15}$$

$(c'_N = c''_N = 0)$.

In studying Na-Ca exchange it is advantageous to carry out the experiments under "zero-*trans*" conditions, i.e., for vanishing sodium concentration on one side and vanishing calcium concentration on the other side. From Eq. (10) the rate of calcium efflux under the condition $c'_N = c''_C = 0$ is predicted to be

$$\Phi'_C = \Phi'_{C,C\to\infty}\frac{c'_C}{c'_C + L'_C} \tag{16}$$

$$\Phi'_{C,C\to\infty} = \frac{k''l'}{q'} \cdot \frac{c''^3_N}{K''_{N1}K''_{N2}K''_{N3}} \tag{17}$$

$$L'_C = K'_C\frac{k''}{q'} \cdot \frac{c''^3_N}{K''_{N1}K''_{N2}K''_{N3}} \tag{18}$$

$$q' \equiv l'\left(1 + \frac{c''_N}{K''_{N1}} + \frac{c''^2_N}{K''_{N1}K''_{N2}} + \frac{c''^3_N}{K''_{N1}K''_{N2}K''_{N3}}\right) + k''\frac{c''^3_N}{K''_{N1}K''_{N2}K''_{N3}} \tag{19}$$

$(c'_N = c''_C = 0)$.

Thus, the half-saturation concentration L'_C as well as the maximum calcium efflux $\Phi'_{C,C\to\infty}$ depend on external sodium concentration c''_N. The calcium influx Φ''_C which is measured under the inverse "zero-*trans*" condition $(c''_N = c'_C = 0)$ is obtained by exchanging the superscripts $'$ and $''$ in Eqs. (16)–(19).

Voltage Dependence of Kinetic Parameters

The voltage dependence of the rate constants is connected with the electric charge which is translocated in the particular reaction step. It has sometimes been assumed that the whole voltage effect on ion fluxes results from the voltage dependence of the rate constants of conformational transitions $\mathrm{Na_3}E' \leftrightarrow E''\mathrm{Na_3}$ and $\mathrm{Ca}E' \leftrightarrow E''\mathrm{Ca}$ in which the ion-loaded binding site is thought to move within the membrane dielectric. Such a situation is possible only as a limiting case. If, as schematically depicted in Fig. 2*A*, the exchanger molecule has wide, low-resistance access channels, nearly the whole voltage drops across the narrow "gating" part of the molecule. In this case the binding sites may move through a substantial part of the transmembrane electric field, whereas entry and release of the ions are little affected by voltage. In general, however, an ion migrating from the aqueous medium to the binding site has to cross a fraction of the electric field, meaning that the equilibrium constant of ion binding becomes voltage dependent (Mitchell, 1969). This situation in which a narrow, high-resistance access channel ("ion well") connects the binding sites to the aqueous medium is depicted in Fig. 2*B*.

In order to describe the voltage dependence of the kinetic constants, we introduce the energy profile of the ion (Na^+ or Ca^{2+}) along the transport pathway, which consists of a series of barriers and

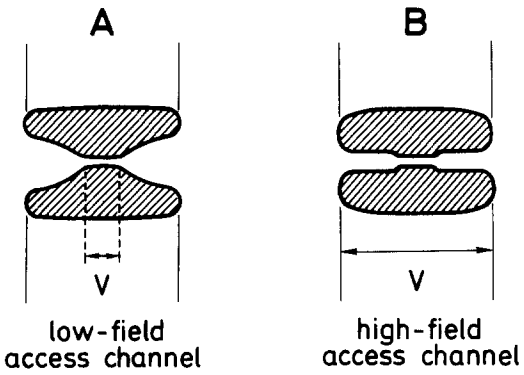

Fig. 2. Two extreme possibilities for the geometry of the access channel connecting the ion-binding sites with the aqueous medium. (*A*) Access channel consisting of a wide, water-filled pore (or vestibule) with a high electrical conductance; the field strength in the access channel is low so that a large fraction of the transmembrane voltage V drops across the narrow "gating" part of the molecule. (*B*) Narrow and ion-specific access channel, allowing the entry of Na^+ and Ca^{2+}, but excluding other ions; the field strength in the access channel is high, so that the rate constants for ion binding and release become voltage dependent ("ion-well" behavior)

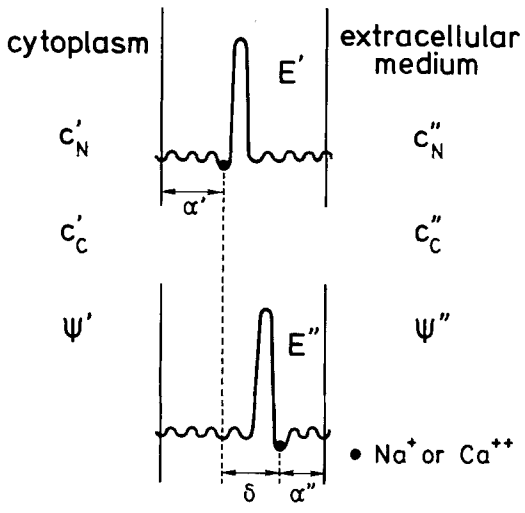

Fig. 3. Energy profile of an ion (Na^+ or Ca^{2+}) along the transport pathway. In conformation E' the ion-binding site is accessible from the cytoplasmic side, but separated from the extracellular medium by a high energy barrier. In conformation E'' the binding site is accessible from the extracellular medium. α', α'' and δ are dielectric distances along the transport pathway

wells (Fig. 3). According to the assumption of fast association-dissociation equilibrium, the ion binding site in conformation E' is connected with the cytoplasmic side by a series of low barriers; it is separated from the extracellular side by a barrier of virtually infinite height. In conformation E'' the barrier toward the cytoplasm is high and the barrier toward the extracellular medium is low. This model of a gated channel has been extensively discussed in the literature (Patlak, 1957; Jardetzky, 1966; Klingenberg et al., 1976; Läuger, 1980, 1985).

If an electrical potential difference, u, exists between the cytoplasm and the extracellular medium [Eq. (4)], a fraction $\alpha' u$ drops between the cytoplasm and the binding site (Fig. 3). The (dimensionless) dielectric distance α' 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 electric field, the equilibrium dissociation constants become voltage dependent:

$$K'_{Ni} = \tilde{K}'_{Ni} \exp(-\alpha' u) \qquad (i = 1,2,3) \tag{20}$$

$$K''_{Ni} = \tilde{K}''_{Ni} \exp(\alpha'' u) \tag{21}$$

$$K'_C = \tilde{K}'_C \exp(-2\alpha' u) \tag{22}$$

$$K''_C = \tilde{K}''_C \exp(2\alpha'' u). \tag{23}$$

$\tilde{K}'_{Ni}$, $\tilde{K}''_{Ni}$, $\tilde{K}'_C$ and $\tilde{K}''_C$ are the values of the equilibrium constants at zero voltage. If the potential of the cytoplasm is positive with respect to the extracellular medium ($u > 0$), the equilibrium constants $1/K'_{Ni}$ of sodium binding at the cytoplasmic site are increased by a Boltzmann factor $\exp(\alpha' u)$.

In the course of the conformational transitions $Na_3E' \rightarrow E''Na_3$ and $CaE' \rightarrow E''Ca$, the binding site together with the bound ion moves, in general, over a certain distance. The electrostatic contribution (in units of kT) to the energy difference between states Na_3E' and $E''Na_3$ is equal to $\delta(z + 3)u$, where δ is the fractional dielectric distance over which the site moves (Fig. 3) and z is the charge on the binding site. Additional charge displacements in the protein may result from reorientation of polar residues other than the ligand groups. If the protein is considered as an assembly of point charges, motion of the j-th charge in the presence of a transmembrane voltage u results in an energy change of magnitude $\eta_j u$, where η_j is the dielectric distance over which the charge moves. This leads to an overall energy contribution ηu, with η being the sum of all η_j (Läuger, 1984). We assume, as an approximation, that the conformational change takes place in a single step which may be described as a transition over a narrow, symmetrical activation barrier. Accordingly, the transition rate constants are given by the rate-theory expressions

$$k' = \tilde{k}' \exp\{[(z + 3)\delta + \eta]u/2\} \tag{24}$$

$$l' = \tilde{l}' \exp\{[(z + 2)\delta + \eta]u/2\}. \tag{25}$$

$\tilde{k}'$ and $\tilde{l}'$ refer to zero voltage. The backward rate

constants k'' and l'' are obtained by reversing the sign of the exponent in Eqs. (25) and (26). From Eqs. (3) and (20)–(25) it is seen that the dielectric coefficients are connected by the following relation:

$$\alpha' + \alpha'' + \delta = 1. \tag{26}$$

Voltage Dependence of Calcium Fluxes

In this section we discuss examples of flux-voltage curves, which are obtained by numerical evaluation of the flux equation. From the shape of the $\Phi(V)$ curve, information on the nature of the voltage-dependent transport steps may be obtained. The results of such an analysis depend on the magnitude of the rate constants and equilibrium constants of the transport system. In the absence of direct binding studies, the equilibrium dissociation constants for Na^+ and Ca^{2+} have to be estimated from the half-saturation constants of fluxes. Assuming that the three sodium sites are identical and independent, the following relations are obtained for the equilibrium dissociation constants K'_{N1}, K''_{N1}, . . .:

$$K'_{N1} = \tfrac{1}{3}K'_N;\ K'_{N2} = K'_N;\ K'_{N3} = 3K'_N \tag{27}$$

$$K''_{N1} = \tfrac{1}{3}K''_N;\ K''_{N2} = K''_N;\ K''_{N3} = 3K''_N. \tag{28}$$

K'_N and K''_N are the intrinsic dissociation constants of Na^+ at the cytoplasmic and at the extracellular side, respectively. The factors $\frac{1}{3}$ and 3 are the usual statistical coefficients describing binding equilibria in a system with multiple binding sites (Tanford, 1961).

Stimulation of calcium efflux from squid giant axons by external sodium is described by a half-saturation concentration of sodium of about 50 mM (Blaustein, 1977). From calcium-influx experiments a similar value is found for the half-saturation concentration of internal sodium (DiPolo, 1979). In the squid axon, the effects of calcium are strongly asymmetric, the half-saturation concentrations for internal and external calcium being of the order of 10 μM and 1 mM, respectively (Baker & Dipolo, 1984; Allen & Baker, 1986*a*). Based on these findings, the following values for the equilibrium dissociation constants will be used in the following:

$$\tilde{K}'_N = \tilde{K}''_N = 50 \text{ mM}$$
$$\tilde{K}'_C = 10\ \mu\text{M};\ \tilde{K}''_C = 1 \text{ mM}.$$

Very little information is available so far on the transition rate constants k', k'', l' and l'' (Fig. 1). From experiments with reconstituted vesicles, Hale et al. (1984) estimated a maximum turnover rate for Na-Ca exchange of about 25 sec^{-1}. This means that at least one of the rate constants must be of the order of 100 sec^{-1} or less. Accordingly, we make the following (rather arbitrary) assignment:

$$\tilde{k}' = 1000 \text{ sec}^{-1};\ \tilde{k}'' = 100 \text{ sec}^{-1}$$
$$\tilde{l}' = 100 \text{ sec}^{-1};\ \tilde{l}'' = 1000 \text{ sec}^{-1}.$$

These values have been chosen such that Eq. (3) accounting for microscopic reversibility is fulfilled.

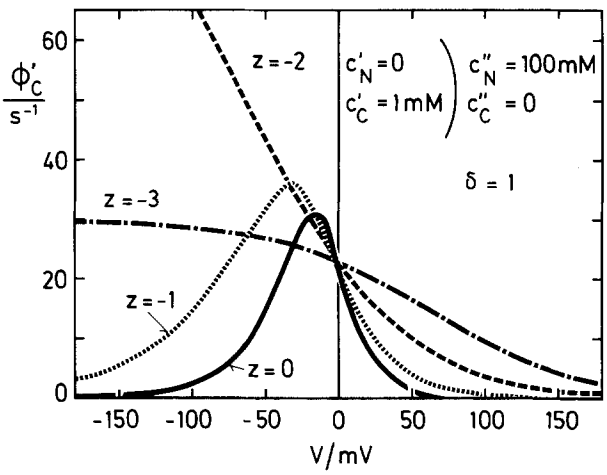

Fig. 4. Calcium efflux Φ'_C [Eq. (10)] as a function of transmembrane voltage V under "zero-*trans*" conditions ($c'_N = c''_C = 0$, $c'_C = 1$ mM, $c''_N = 100$ mM) for different values of the valency z of the ligand system. Φ'_C is referred to a single exchanger molecule. The following parameter values have been used for the calculation of Φ'_C: $\tilde{K}'_N = \tilde{K}''_N = 50$ mM, $\tilde{K}'_C = 10\ \mu$M, $\tilde{K}''_C = 1$ mM, $\tilde{k}' = 1000\ sec^{-1}$, $\tilde{k}'' = 100\ sec^{-1}$, $\tilde{l}' = 100\ sec^{-1}$, $\tilde{l}'' = 1000\ sec^{-1}$, $\eta = \alpha' = \alpha'' = 0$, $\delta = 1$. The condition $\delta = 1$ corresponds to a low-field access channel, as shown in Fig. 2*A*

In the following we consider "zero-*trans*" experiments under the condition $c'_N = c''_C = 0$. "Zero-*trans*" experiments have the advantage that under the condition $c'_N = c''_C = 0$ (or $c''_N = c'_C = 0$) the equations relating the observable fluxes with the kinetic parameters considerably simplify. For $c'_N = c''_C = 0$ the net calcium-flux Φ_C [Eq. (11)] becomes equal to the unidirectional calcium-efflux Φ''_C [Eq. (10)]. The numerical simulations of Φ'_C which will be discussed in the following therefore equally apply to the net flux Φ_C as well to the electric current $I = -e_o\Phi_C$ measured under "zero-*trans*" conditions.

The voltage dependence of Φ'_C has been evaluated for different sets of values of the dielectric coefficients α', α'' and δ and of the charge z of the ligand system (the dielectric coefficient η describing intrinsic charge displacements in the protein is set equal to zero throughout). Figure 4 represents flux-voltage curves under the condition $c'_N = c''_C = 0$, $c'_C = 1$ mM, $c''_N = 100$ mM for different values of z (z

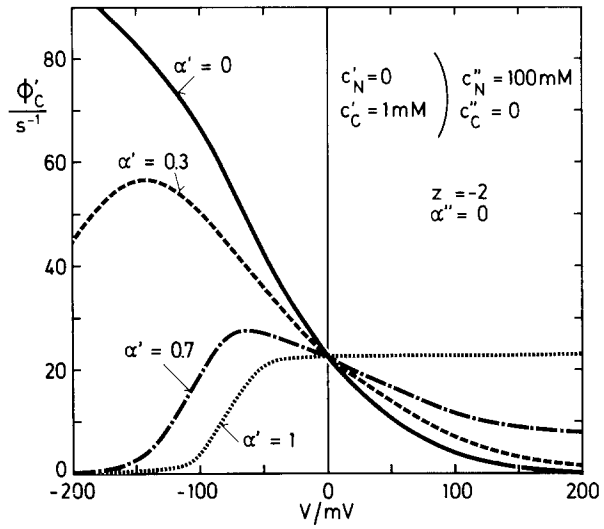

Fig. 5. Calcium efflux Φ'_C as a function of membrane potential V for different values of the dielectric distance α' between the cytoplasmic binding site and the cytoplasmic membrane surface (Fig. 3). For $\alpha' > 0$ the equilibrium constant of calcium binding becomes voltage dependent. This "calcium-well" effect leads to a decrease of calcium efflux at large inside-negative membrane potentials. $z = -2$, $\alpha'' = 0$, $\delta = 1 - \alpha'$. The other parameters were the same as in Fig. 4. For $\alpha' = 0$, Φ'_C approaches a limiting value of 100 sec^{-1} at large negative potentials

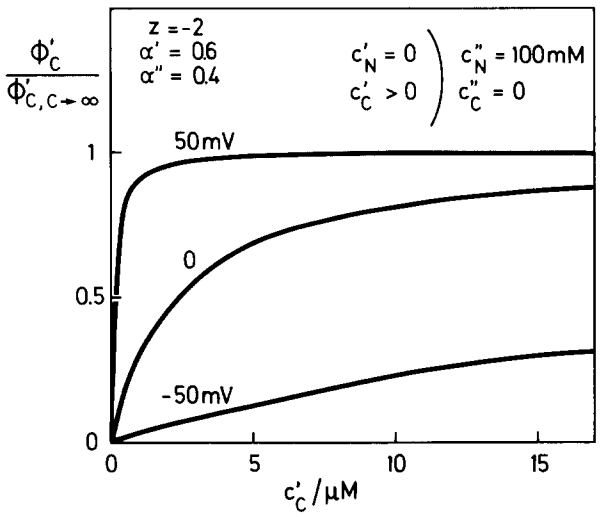

Fig. 6. Calcium efflux Φ'_C as a function of cytoplasmic calcium concentration c'_C for different membrane potentials. Φ'_C is referred to the maximum flux $\Phi'_{C,C\to\infty} \equiv \Phi'_C(c'_C \to \infty)$. $z = -2$, $\alpha' = 0.6$, $\alpha'' = 0.4$, $\delta = 0$; the other parameters were the same as in Fig. 4

$= 0, -1, -2, -3$). For $z = 0$ and $z = -1$ the net calcium flux Φ'_C exhibits a biphasic shape, approaching zero for large negative voltages. This nonmonotonic behavior results from the voltage dependence of the transition rate constants (Eisner & Lederer, 1985; Johnson & Kootsey, 1985). For $z = 0$ and $z = -1$ both the sodium-loaded form Na_3E and the calcium-loaded form CaE bear a net positive charge, so that the two conformational transitions ($Na_3E'' \to E'Na_3$ and $E'Ca \to CaE''$) exhibit opposite voltage dependence. This means that at large positive or large negative voltage one of the transitions is inhibited by the electric field. In contrast, for $z = -2$ and $z = -3$ one of the ion-loaded forms (Na_3E or CaE) is electrically neutral. The corresponding conformational transition is independent of voltage and becomes rate limiting at large driving force. This results in a saturating behavior for $V \to -\infty$ as indicated by Fig. 4 (for $z = -2$ the limiting value for $V \to -100$ mV is $\Phi'_C = 100$ sec^{-1}).

In Fig. 5 flux-voltage curves are represented for the case that ion binding is voltage dependent. Under the condition $\alpha' > 0$, an inside-negative voltage ($V < 0$) increases the equilibrium dissociation constant of calcium at the cytoplasmic binding site. This "calcium-well" effect leads to a decrease of net calcium efflux at large negative membrane potentials.

Detailed information on the voltage-dependent behavior of the exchanger may be obtained by studying the concentration dependence of fluxes at different membrane potentials. This is illustrated in Fig. 6 in which the rate of calcium efflux Φ'_C is plotted as a function of internal calcium concentration c'_C. In the example considered in Fig. 6 it is assumed that charge translocation associated with the E'/E'' transition is negligible ($\delta = 0$), and that the main voltage effect results from the field dependence of ion binding (α', $\alpha'' > 0$). This corresponds to the situation depicted in Fig. 2*B* (high-field access channel). It is seen from Fig. 6 that the calcium concentration at which calcium efflux is half-maximal strongly depends on membrane potential. At inside-positive potentials (calcium binding enhanced) the half-saturation concentration is much lower than at inside-negative potentials.

In Figs. 7 and 8 the half-saturation concentration L'_C of cytoplasmic calcium and the maximum flux $\Phi'_{C,C\to\infty} \equiv \Phi'_C(c'_C \to \infty)$, as obtained from Eqs. (17) and (18), are plotted for different values of α' and $\alpha'' = 1 - \alpha'$. The half-saturation concentration L'_C strongly depends on membrane potential V, as seen from Fig. 7. This is a consequence of the combined effects of voltage on the binding of calcium and sodium at the cytoplasmic and extracellular sites. The voltage dependence of $\Phi'_{C,C\to\infty}$ (Fig. 8) exclusively results from the "sodium-well" effect at the extracellular binding site, since for $c'_C \to \infty$ the calcium site is always occupied.

In an analogous way, the calcium efflux may be studied as a function of external sodium concentra-

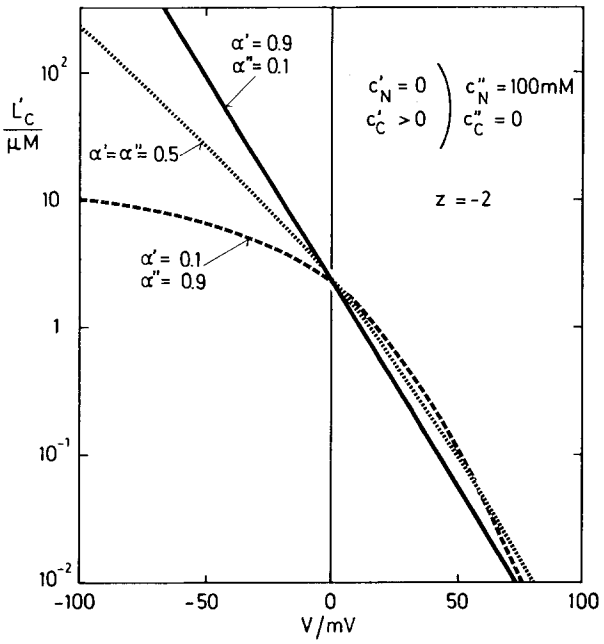

Fig. 7. Half-saturation concentration L'_C of cytoplasmic calcium as a function of membrane potential V for different values of α' and $\alpha'' = 1 - \alpha'$. L'_C was calculated from Eq. (18) with $\delta = 0$ and $z = -2$; the other parameters were the same as in Fig. 4. The voltage dependence of L'_C results from the combined effects of membrane potential on the binding of calcium and sodium at the cytoplasmic and extracellular sites, respectively

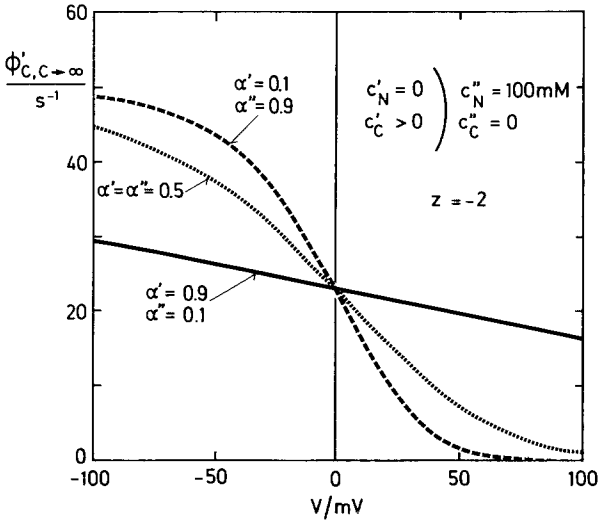

Fig. 8. Maximum calcium efflux $\Phi'_{C,C\to\infty} \equiv \Phi'_C(c'_C \to \infty)$ as a function of membrane potential V for different values of α' and $\alpha'' = 1 - \alpha'$. $\Phi'_{C,C\to\infty}$ was calculated from Eq. (17) using the same set of parameters as in Fig. 7. The voltage dependence of $\Phi'_{C,C\to\infty}$ results from the sodium-well'' effect at the extracellular binding site

tion c''_N at constant c'_C and different membrane potentials V. The maximum flux $\Phi'_{N,C\to\infty}$ and the half-saturation concentration L''_N for external sodium contain additional independent information on the nature of the voltage-dependent steps.

Simultaneous Mechanism

In the simplest version of the simultaneous mechanism (Fig. 9) the exchanger in conformation E' binds three sodium ions at the cytoplasmic side and a calcium ion at the extracellular side. After a conformational transition $Na_3E'Ca \rightarrow CaE''Na_3$, the sodium ions are released to the extracellular medium and the calcium ion to the cytoplasm. The original state is restored by the transition $E'' \rightarrow E'$ in the unloaded form of the molecule. For the calculation of the steady-state fluxes we introduce the following assumptions:

a) Conformational transitions are possible only in the empty and in the fully loaded form of the exchanger (E and Na_3ECa).

b) Binding and release reactions are not rate limiting.

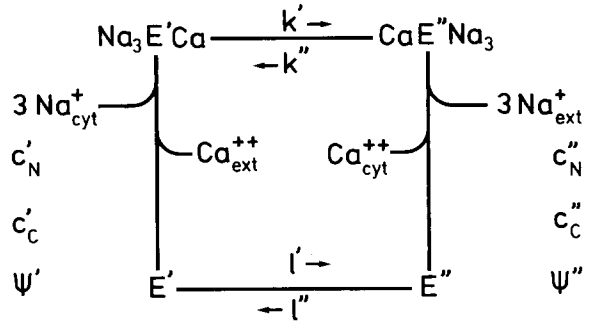

Fig. 9. Simultaneous mechanism for countertransport of Na^+ and Ca^+. In conformation E' the exchanger binds three sodium ions at the cytoplasmic side and a calcium ion at the extracellular side. After a conformational transition $Na_3E'Ca \rightarrow CaE''Na_3$, the sodium ions are released to the extracellular medium and the calcium ion to the cytoplasm

c) The three binding sites for Na^+ are equivalent.

d) The sodium sites do not bind calcium and vice versa.

e) Na^+ and Ca^{2+} bind independently of each other.

Using the same notation as above, the binding equilibria may be described by the relations

$$\frac{K'_{N1}}{c'_N} = \frac{x[E']}{x[NaE']} = \frac{x[E'Ca]}{x[NaE'Ca]} \tag{29}$$

$$\frac{K'_{N2}}{c'_N} = \frac{x[NaE']}{x[Na_2E']} = \frac{x[NaE'Ca]}{x[Na_2E'Ca]} \tag{30}$$

$$\frac{K'_{N3}}{c'_N} = \frac{x[Na_2E']}{x[Na_3E']} = \frac{x[Na_2E'Ca]}{x[Na_3E'Ca]} \tag{31}$$

$$\frac{K''_C}{c''_C} = \frac{x[E']}{x[E'Ca]} = \frac{x[NaE']}{x[NaE'Ca]} = \frac{x[Na_2E']}{x[Na_2E'Ca]} = \frac{x[Na_3E']}{x[Na_3E'Ca]}. \tag{32}$$

Analogous relations hold for state E''. The rate constants k', k'', l', l'' and the equilibrium constants K'_{N1}, K'_{N2}, . . . are again connected by Eq. (3). The steady-state fluxes may be obtained as described above. The result reads:

$$\Phi'_N = \frac{3k'}{\sigma} \cdot \frac{c'^3_N c''_C}{K'_{N1}K'_{N2}K'_{N3}K''_C}\left[l'' + k'' \frac{c''^3_N c'_C}{K''_{N1}K''_{N2}K''_{N3}K'_C}\right] \tag{33}$$

$$\Phi'_C = \frac{k''}{\sigma} \cdot \frac{c''^3_N c'_C}{K''_{N1}K''_{N2}K''_{N3}K'_C}\left[l' + k' \frac{c'^3_N c''_C}{K'_{N1}K'_{N2}K'_{N3}K''_C}\right] \tag{34}$$

$$\Phi_C = -\frac{1}{3}\Phi_N = \frac{1}{\sigma} \cdot \frac{k''l'}{K''_{N1}K''_{N2}K''_{N3}K'_C}[c''^3_N c'_C - c'^3_N c''_C \exp(u)] \tag{35}$$

$$\sigma \equiv R'S'' + R''S' \tag{36}$$

$$R' \equiv \left(1 + \frac{c''_C}{K''_C}\right)\left(1 + \frac{c'_N}{K'_{N1}} + \frac{c'^2_N}{K'_{N1}K'_{N2}} + \frac{c'^3_N}{K'_{N1}K'_{N2}K'_{N3}}\right) \tag{37}$$

$$S' \equiv l' + k' \frac{c'^3_N c''_C}{K'_{N1}K'_{N2}K'_{N3}K''_C}. \tag{38}$$

Φ''_N, Φ''_C, R'' and S'' are obtained from Φ'_N, Φ'_C, R' and S' by exchanging the superscripts $'$ and $''$.

When the calcium efflux is measured under "zero-*trans*" conditions with $c'_N = c''_C = 0$, Eq. (34) assumes the form

$$\Phi'_C = \Phi'_{C,C\to\infty} \frac{c'_C}{c'_C + L'_C} \tag{39}$$

$$\Phi'_{C,C\to\infty} = \frac{k''l'}{q'} \cdot \frac{c''^3_N}{K''_{N1}K''_{N2}K''_{N3}} \tag{40}$$

$$L'_C = \frac{K'_C}{q'}\left[l'\left(1 + \frac{c''_N}{K''_{N1}} + \frac{c''^2_N}{K''_{N1}K''_{N2}} + \frac{c''^3_N}{K''_{N1}K''_{N2}K''_{N3}}\right) + l''\right]. \tag{41}$$

The quantity q' is given by Eq. (19). As will be discussed below, the dependence of L'_C on c''_N may be used as an experimental criterion for distinguishing between consecutive and simultaneous reaction schemes.

The voltage dependence of the kinetic constants may be evaluated in a similar way as described for the consecutive mechanism. Since in the simultaneous model the geometry of the calcium and sodium binding sites may be different, we introduce separate quantities α'_N and α'_C for the dielectric distances of the sodium and calcium sites at the cytoplasmic face of the membrane (*compare* Fig. 3). In an analogous way, quantities α''_N, α''_C, δ_N, δ_C, z_N and z_C are defined instead of α'', δ and z. In analogy to Eqs. (20)–(26) the voltage dependence of the kinetic constants is then described by the relations

$$K'_{Ni} = \tilde{K}'_{Ni} \exp(-\alpha'_N u) \qquad (i = 1,2,3) \tag{42}$$

$$K''_{Ni} = \tilde{K}''_{Ni} \exp(\alpha''_N u) \tag{43}$$

$$K'_C = \tilde{K}'_C \exp(-2\alpha'_C u) \tag{44}$$

$$K''_C = \tilde{K}''_C \exp(2\alpha''_C u) \tag{45}$$

$$k' = \tilde{k}' \exp\{[(z_N + 3)\delta_N - (z_C + 2)\delta_C + \eta]u/2\} \tag{46}$$

$$l' = \tilde{l}' \exp[(z_N\delta_N - z_C\delta_C + \eta)u/2] \tag{47}$$

$$3\alpha'_N - 2\alpha'_C + 3\alpha''_N - 2\alpha''_C + 3\delta_N - 2\delta_C = 1. \tag{48}$$

k'' and l'' are obtained by reversing the sign of the exponent in Eqs. (46) and (47).

Distinction between Simultaneous and Consecutive Mechanism

Simultaneous as well as consecutive reaction schemes have been discussed in the literature as possible mechanisms of Na-Ca exchange (Blaustein, Russel & DeWeer, 1974; Blaustein, 1977; Mullins, 1977; Wong & Bassingthwaighte, 1981; Eisner & Lederer, 1985; Johnson & Kootsey, 1985). Both mechanisms may be distinguished by the concentration dependence of fluxes. Ledvora and Hegyvary (1983) studied calcium influx into cardiac vesicles as a function of the concentrations of extravesicular calcium (c''_C) and intravesicular sodium (c'_N)*. From the finding that the half-saturation concentration L''_C of external calcium was independent of c'_N, they argued that the exchanger functions by a simultaneous mechanism. This conclusion is not justified, however. It can be seen from Eq. (41) that for the simultaneous mechanism, L''_C depends, in general, on c'_N (L''_C is obtained from Eq. (41) by exchanging the superscripts $'$ and $''$). The condition that L''_C is independent of c'_N is met only in certain limiting cases. The same is true for the consecutive mechanism, as Eq. (18) shows. This means that the observed independence of L''_C on c'_N is compatible with both the consecutive and the simultaneous mechanism. (In the consecutive mechanism, L''_C becomes independent of c'_N for $k'm'_1m'_2m'_3 \gg l''(1 + m'_1 + m'_1m'_2 + m'_1m'_2m'_3)$, where $m_i \equiv c'_N/K'_{Ni}$). A similar argument applies to the finding that the half-saturation concentration L''_N of external sodium is inde-

* In identifying the intravesicular sodium concentration with the cytoplasmic concentration c'_N of Na^+ (and the extravesicular calcium concentration with c''_C) we neglect the fact that the vesicle preparation contains a mixture of right-side-out and inside-out vesicles (Bers, Philipson & Nishimoto, 1980; Philipson, 1985).

pendent of internal calcium concentration in the squid axon (Blaustein, 1977).

The difficulties encountered in the experimental distinction between simultaneous and consecutive models for Na-Ca exchange have already been discussed by Johnson and Kootsey (1985). These authors preferred a simultaneous model on the basis of the finding that Ca-Ca exchange requires the presence of monovalent cations. It has been shown, however, that in activating Ca-Ca exchange, K^+ is more effective than Na^+, whereas K^+ is a poor substitute for Na^+ in the normal Na-Ca exchange mode of the transport system (Ledvora & Hegyvary, 1983; Philipson, 1985). This is consistent with the observation that the activating monovalent cation is not transported (Slaughter, Sutko & Reeves, 1983).

The available experimental evidence thus seems not sufficient for excluding one of the two mechanisms. A straightforward experimental distinction between simultaneous and consecutive models is possible by studying the concentration dependence of fluxes under "zero-*trans*" conditions. As a comparison of Eqs. (18) and (41) shows, the two mechanisms differ in the dependence of half-saturation concentration L'_C on extracellular sodium concentration c''_N. L'_C is predicted to vanish for $c''_N \to 0$ in the consecutive mechanism, but approaches a finite value $K'_C(1 + l''/l')$ for $c''_N \to 0$ in the simultaneous mechanism. An analogous statement applies when L''_C is measured as a function of c'_N. The method of measuring the half-saturation concentration of one substrate as a function of the concentration of the second substrate has already been used in the case of sodium-coupled cotransport for distinguishing between consecutive and simultaneous mechanisms (Kessler & Semenza, 1983; Restrepo & Kimmich, 1985; Jauch & Läuger, 1986).

Comparison with Experimental Results

Voltage dependence and current-generating capacity of the Na-Ca exchange system are well documented in the literature (Brinley & Mullins, 1974; Blaustein et al., 1974; Mullins & Brinley, 1975; DiPolo, 1979; Mullins, 1979; Sjodin & Abercrombie, 1978; Bers et al., 1980; Caroni et al., 1980; Philipson & Nishimoto, 1980; Reeves & Sutko, 1980; Lamers & Stinis, 1981; Lederer & Nelson, 1983; Ledvora & Hegyvary, 1983; Mullins et al., 1983; Nelson & Lederer, 1984; Reeves & Hale, 1984; DiPolo et al., 1985; Kimura et al., 1986; Allen & Baker, 1986*a,b*; Kimura et al., 1987). Voltage effects on Na-Ca exchange could only recently be analyzed in more quantitative terms. Ledvora and Hegyvary (1983) measured the rate Φ''_C of sodium-dependent calcium influx into heart-sacrolemma vesicles at 37°C and observed, for voltages $V \equiv \psi_i - \psi_o$ between −60 and +140 mV, an exponential relationship of the form

$$\Phi''_C = \tilde{\Phi}''_C \exp(V/92\ \text{mV}) = \tilde{\Phi}''_C \exp(0.29\ u) \qquad (49)$$

(at an intravesicular Na^+ concentration of 150–160 mM and an extravesicular calcium concentration of 50 μM). A similar form of the voltage dependence of sodium-calcium exchange current has recently been reported by Kimura et al. (1987).

It has sometimes been assumed that a transport system which translocates a single net-charge per cycle should exhibit a voltage dependence corresponding to an e-fold change of transport rate for a voltage change of $kT/e_o \approx 25$ mV. This, however, is only true under special conditions and only in a limited voltage range. For instance, according to Eq. (17), the calcium influx under "zero-*trans*" conditions at saturating concentrations of Na^+ and Ca^{2+} is given by

$$\Phi''_C \equiv \Phi''_{C,\infty} = \frac{k'l''}{k' + l''} \qquad (50)$$

$(c''_N = c'_C = 0; \quad c'_N, c''_C \to \infty).$

Depending on the magnitude of the parameters z, δ and h in Eqs. (24) and (25), the voltage dependence of $\Phi''_{C,\infty}$ can differ widely. If δ and h both vanish, $\Phi''_{C,\infty}$ becomes voltage independent at any value of z. On the other hand, for $z = -2$, $\eta = 0$ and $\delta = 1$ (low-field access channel) one obtains

$$\Phi''_{C,\infty} = \frac{\tilde{k}' + \tilde{l}''}{\tilde{\Phi}''_{C,\infty}\,\tilde{k}' \exp(u/2) + \tilde{l}''} \exp(u/2). \qquad (51)$$

For $\tilde{l}'' \gg \tilde{k}'$ the flux is proportional to $\exp(u/2) \approx \exp(V/50\ \text{mV})$. In summary, it is clear that a result of the form of Eq. (49) is compatible with the translocation of a single net charge per cycle.

Allen and Baker (1986*a,b*) studied the voltage dependence of unidirectional inward and outward fluxes of calcium in the squid axon (the inward flux was obtained from the coupled sodium efflux). The outward flux Φ'_C monotonously decreased and the inward flux Φ''_C monotonously increased in magnitude with increasing (inside positive) membrane potential. The net outward flux $\Phi_C = \Phi'_C - \Phi''_C$ reversed its sign near −40 mV and exhibited an approximately linear voltage dependence between −90 and +10 mV. According to Figs. 4 and 5, the monotonous behavior of Φ'_C is compatible with a

valency $z = -2$ of the ligand system and with shallow ion wells (α' and α'' small). It is obvious that studies of this kind will yield detailed mechanistic information, provided that the flux-voltage relationship of the exchanger can be measured in a wide range of intra- and extracellular ion concentrations.

Discussion

The foregoing analysis of voltage effects on Na-Ca exchange has been based on two microscopic reaction models which should be considered as minimum models. Using the assumption that binding and release of ions are not rate limiting, the equations relating fluxes to ion concentrations and voltage could be obtained in closed form. The voltage dependence of ion fluxes can be expressed by a set of "dielectric coefficients" describing the magnitude of charge translocation associated with individual reaction steps. As seen from the numerical examples represented in Figs. 4 and 5, flux-voltage curves can exhibit a variety of different shapes; from the form of the Φ–V relationship inferences can be made on the charge ze_o of the ligand system and on the magnitude of the dielectric coefficients. A quantitative evaluation of the dielectric coefficients requires the knowledge of the rate constants and equilibrium binding constants of the transport system. In the absence of such detailed information, qualitative conclusions on the nature of the charge-translocating reaction steps are still possible. For instance, if (in the consecutive model) the ligand valency z is zero or -1, the flux-voltage curve is always nonmonotonic (Fig. 4), since under this condition the calcium-loaded form CaE as well as the sodium-loaded form Na_3E of the exchanger bear a positive charge; this leads to an inhibition of one of the conformational transitions at large (positive or negative) voltages.

Another general prediction concerns the concentration dependence of fluxes. If part of the transmembrane voltage drops between binding site and aqueous solution (α', $\alpha'' > 0$ in Fig. 3), the equilibrium constant of ion binding becomes a function of voltage. Since Na^+ and Ca^{2+} bind from opposite sides, the half-saturation concentrations of the two ions are inversely affected by voltage. For a detailed kinetic analysis of the transport system, it is therefore important to measure the voltage dependence of fluxes in a wide range of sodium and calcium concentrations. It is preferable to carry out these experiments under "zero-*trans*" conditions ($c'_N = c''_C = 0$ or $c''_C = c'_N = 0$), since in this case the theoretical relations for the concentration and voltage dependence of fluxes considerably simplify [*compare* Eqs. (16)–(19) and (39)–(41)].

Additional information may be obtained by studying Ca-Ca exchange mediated by the Na-Ca transport system. Although this transport mode consisting in a one-for-one exchange is nonelectrogenic, the rate of transport may nevertheless depend on voltage. This is directly seen from Eq. (15), which represents the unidirectional calcium flux Φ'_C as a function of the equilibrium dissociation constants K'_C and K''_C and of the transition rate constants l' and l''. Since l', l'', K'_C and K''_C may be affected by the transmembrane electric field, Φ'_C will, in general, depend on voltage. A particularly simple situation is given in the presence of saturating calcium concentrations ($c'_C \gg K'_C$, $c''_C \gg K''_C$). In this case the voltage dependence of Φ'_C results exclusively from the voltage dependence of l' and l''.

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