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The Kinetics of Ca-Na Exchange in Excitable Tissue

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Abstract

A model is proposed to describe the Na-Ca exchange in excitable tissues. The present scheme requires a carrier mechanism that exchanges 3Na for 1Ca across the membrane under the electrochemical gradient of Na. The carriers, assumed to be trivalent anions, have monovalent and divalent sites; Ca and Na can compete only at the second site. The partially and fully loaded carrier-ion complexes are mobile and diffusible across the membrane. Subsequently, analytical expressions for Na and Ca unidirectional flux at steady state are derived in terms of intracellular concentration (Na_i and Ca_i) and extracellular concentration (Na_o and Ca_o) as well as membrane potential, E_M . Published experimental flux data on cardiac muscle, squid axon, and rat synaptosomes can be satisfactorily fitted with the flux equation simply by adjusting the numerical constants.

1. INTRODUCTION

Regulation of intracellular calcium concentration (Ca_i) by passive equilibration in a cell with a resting potential of -60 mV would result in Ca_i being three orders of magnitude higher than the extracellular calcium concentration (Ca_o). However, both direct and indirect measurements indicate that Ca_i is significantly less than Ca_o . In cardiac muscle, for example, the actual ionized Ca_i is about 10^{-6} to 10^{-7} M, while Ca_o is 10^{-2} to 10^{-3} M [15,23]. In the squid axon, with an internal potential of -50 to -60 mV and Ca_o of 11 mM, the ionized Ca concentration in axoplasm should be about 1 M. Experimental evidence [4], however, shows that the ionized Ca is less than $0.3 \mu M$. The low level of Ca_i implies that Ca ions are actively extruded from the cell.

Lüttgau and Niedergerke [18] observed the interaction between sodium and calcium in frog heart and determined that it was a means of establishing and maintaining calcium homeostasis. They also showed that even in depolarized cardiac muscle, contraction could be effected by depleting Na_o . The contracture in Na-poor media [22] could account for a marked increase in Ca influx and a net gain of Ca. Prompt relaxation occurred [22,27] in conjunction with a net loss of Ca when Ca-loaded ventricles were returned to control Ringers. To explain these results, Niedergerke [22] postulated that Ca entry occurred *via* a carrier mechanism for which Na_o^+ and Ca_o^{2+} ions competed, presumably in the ratio of 2 : 1.

Reuter and associates [16,24] examined the effect of Na_o and Na_i on Ca fluxes in mammalian cardiac muscle. As in frog heart, partial or complete replacement of Na_o increased the Ca influx and the net Ca content of the tissue. The Ca efflux from guinea pig auricles was found to depend on both Na_o and Ca_o and was almost linearly proportional to $[Ca_o]/[Na_o]^2$ in a semilog plot. These investigators suggested that Na_o and Ca_o compete for external binding sites on a Ca carrier in the ratio of 2 : 1.

Recently Jundt et al. [17], using KCl to depolarize the guinea pig auricle, observed that the Na-activated Ca efflux was independent of membrane potential; this implies an electroneutrality for the Na-Ca exchange mechanism.

Substantial studies of Ca-Na exchange on squid axon and other preparations (barnacle muscle, synaptosomes) have been conducted by Baker and associates [1–5] and Blaustein and associates [6–12]. Detailed data from these studies indicate that the stoichiometry of the exchange is most likely to be 3Na^+ entering for one Ca^{2+} leaving, despite a suggested ratio of 4 : 2 [19].

If more than two Na^+ ions couple against one Ca^{2+} ion, the exchange mechanism is electrogenic, because it is dependent on the membrane potential. Blaustein et al. [9] showed the inhibition of Ca efflux by increasing membrane potential. Mullins and Brinley [20] demonstrated that hyperpolarization increased and depolarization reduced Ca efflux of squid axon. Although Baker and McNaughton [5] found little effect on Ca efflux by hyperpolarization, Requena et al. [25] depolarized the membrane of squid axon and found an increase of intracellular calcium; this implied an elevation of Ca influx and a decrease of Ca efflux. The flux data on squid axon therefore clearly indicate that the exchange ratio of Na to Ca is more than 2: 1.

Based on the experimental data of Brinley et al. [13] and Requena et al. [25], Mullins [21] recently proposed a model for Na/Ca transport in squid axon. His model required the binding of 4Na to a carrier before a Ca binding site was induced. Although unidirectional flux equations were derived, Na or Ca fluxes were not calculated or compared with experimental data.

Sidell and Bassingthwaighte [26] proposed a model of Na-Ca exchange, assuming an exchange ratio of 2 : 1. While their model was able to predict the observed flux data on cardiac muscle fiber and on beating rat hearts, the kinetic data on nerve, squid axon, or synaptosomes were difficult to describe.

We have modified the model of Sidell and Bassingthwaighte to account for an exchange ratio of 3 : 1, in order to quantify the flux data of excitable tissues.

2. THE Na-Ca CARRIER MODEL

2.1. CHARACTERISTICS AND ASSUMPTIONS

The present scheme of Na-Ca exchange is defined in Fig. 1. It basically evolved from the proposal of Blaustein et al. [9]. The following is a brief description of the model characteristics.

1. The model involves three phases: intracellular, membrane, and extracellular. Carrier is confined to the membrane, and sodium and calcium to the intracellular and extracellular phases except when combined with carrier.
2. The carriers are triple anions and so have binding sites for Na and Ca.
3. The first site is monovalent and can bind a single Na ion; the second site is divalent with specificity for either 2Na^+ or 1Ca^{2+} .
4. Ca and Na can compete at the second site but not at the first site.
5. When an alkali metal ion or dextrose AM_o substitutes for Na_o , such $\text{AM}_o + \text{Na}_o = \text{constant}$; the $\text{Na}_o^2/K_{o\text{Na}}$ in the divalent site is replaced by $(\text{AM}_o/\sqrt{K_{\text{AM}}} + \text{Na}_o/\sqrt{K_{o\text{Na}}})^2$, where K_{AM} is the binding constant of AM_o .

6. The equilibration between carrier and cations is assumed to be extremely rapid, so that the fractions of free carrier and of carrier bound to Na and Ca are governed by the concentrations of ions in the solution and not by the transmembrane fluxes.
7. Only the carrier-ion complexes are diffusible across the membrane. Free carrier is assumed to diffuse slowly or not at all.
8. The unidirectional flux of J_i of the i th cation is assumed to be proportional to its carrier-cation concentration S_i :

$$J_i = P_s S_i,$$

where P_s is the transport rate of S_i .

Our model assumes free carriers on both sides of the membrane, having binding sites for Na and Ca with asymmetric binding constants. At steady state, the transmembrane fluxes depend only on the concentrations of carrier-cation complexes and on the ionic concentrations at the surface of origin. These fluxes do not require any sequential movements of the translocated ions. The activation on one side of the membrane is independent of saturation on the other side. Subsequently, the efflux and influx of Na and Ca in Na-Ca, Ca-Na, Ca-Ca, and Na-Na exchange can occur simultaneously.

Although the exchange scheme depicted in Fig. 1 is not specifically designed for simultaneous exchange (as the recent schematic model of Blaustein [12] is), it is not a sequential exchange model. Our model is more appropriate for simultaneous than sequential exchange because of its characteristics and the assumptions we have made.

2.2. THE KINETICS

Flux studies indicate transient change of fluxes due to a step change in the external environment such as an abrupt increase or reduction of Na_o or Ca_o . This transient could be caused by the transitional change of carrier concentration on either side of the membrane. In other words, if Y_i is the fraction of total carrier on the inside of the membrane, then the rate of change of Y_i is

$$\frac{dY_i}{dt} = \alpha(1 - Y_i) - \beta Y_i.$$

At steady state, $dY_i/dt = 0$, and

$$Y_{i,ss} = \frac{\alpha}{\alpha + \beta}, \quad (1)$$

where α and β are functions of transport rate and internal and external ionic concentrations.

Most flux data were obtained during steady state. Hence, only Eq. (1) is used.

Let C_i and C_o be the concentrations of free carriers on both sides of the membrane. The unidirectional flux of all free carriers as well as the carrier-cation complexes is equal to the sum of all the products of transport rate times the concentrations. The carrier influx J_{oiC} is

$$J_{oiC} = P_C C_o + P_{Ca} Ca C_o + P'_{Ca} Ca Na C_o + P_{Na} Na_2 C_o + P'_{Na} Na_3 C_o.$$

Using the equilibrium binding equation which defines the binding constants, and factoring out C_o , J_{oiC} becomes

$$J_{oiC} = C_o \left(P_c + \frac{P_{Ca} Ca_o}{K_{oCa}} + \frac{P'_{Ca} Ca_o \cdot Na_o}{K''_{oCa}} + \frac{P_{Na} Na_o^2}{K_{oNa}} + \frac{P'_{Na} Na_o^3}{K''_{oNa}} \right),$$

and

$$J_{oiC} = C_o \gamma_o,$$

where γ_o represents the terms inside parentheses.

The carrier efflux J_{ioC} is given by an analogous expression:

$$J_{ioC} = C_i \gamma_i,$$

where γ_o , γ_i can be regarded as the concentration-dependent overall transport rates.

As carrier is conserved in the present scheme, the total carrier concentration, C_T , free or complex, is constant:

$$\begin{aligned} C_T &= C_i + CaC_i + CaNaC_i + Na_2C_i + Na_3C_i + C_o + CaC_o + CaNaC_o + Na_2 \cdot C_o + Na_3C_o, \\ &= C_i \left(1 + \frac{Ca_i}{K_{iCa}} + \frac{Ca_i Na_i}{K''_{iCa}} + \frac{Na_i^2}{K_{iNa}} + \frac{Na_i^3}{K''_{iNa}} \right) + C_o \left(1 + \frac{Ca_o}{K_{oCa}} + \frac{Ca_o Na_o}{K''_{oCa}} + \frac{Na_o^2}{K_{oNa}} + \frac{Na_o^3}{K''_{oNa}} \right), \end{aligned}$$

or

$$C_T = C_i \delta_i + C_o \delta_o, \quad (2)$$

where δ_i and δ_o replace the bracketed terms and are considered as concentration-distributed factors.

During steady state, the net flux of carriers and carrier-ion complexes must be zero, implying

$$J_{ioC} = J_{oiC},$$

or

$$C_i \gamma_i + C_o \gamma_o = 0. \quad (3)$$

Equations (2) and (3) give

$$C_o = \frac{C_T \gamma_i}{\gamma_i \delta_o + \gamma_o \delta_i}, \quad (4)$$

$$C_i = \frac{C_T \gamma_o}{\gamma_i \delta_o + \gamma_o \delta_i}. \quad (5)$$

Equation (2) can be written as

$$\begin{aligned} I &= \frac{C_i \delta_i}{C_T} + \frac{C_o \delta_o}{C_T}, \\ &= Y_i + Y_o. \end{aligned} \quad (6)$$

Therefore, in Eq. (1),

$$\begin{aligned} \alpha &= \frac{\gamma_i \delta_o}{\gamma_i \delta_o + \gamma_o \delta_i}, \\ \beta &= \frac{\gamma_o \delta_i}{\gamma_i \delta_o + \gamma_o \delta_i}. \end{aligned} \quad (7)$$

The amount of carrier complex can be described by substituting the expression for C_o and C_i from Eqs. (4) and (5) into the mass action expression for binding:

$$\begin{aligned} \text{Ca}C_i &= \frac{C_i C_{a_i}}{K_{iCa}} = C_T \frac{\gamma_o}{\gamma_i \delta_o + \gamma_o \delta_i} \frac{C_{a_i}}{K_{iCa}}, \\ \text{CaNa}C_i &= \frac{C_i C_{a_i} \text{Na}_i}{K_{iCa}''} = C_T \frac{\gamma_o}{\gamma_i \delta_o + \gamma_o \delta_i} \frac{C_{a_i} \text{Na}_i}{K_{iNa}''}, \\ \text{Na}_2C_i &= \frac{C_i \text{Na}_i^2}{K_{iNa}^2} = C_T \frac{\gamma_o}{\gamma_i \delta_o + \gamma_o \delta_i} \frac{\text{Na}_i^2}{K_{iNa}^2}, \\ \text{Na}_3C_i &= \frac{C_i \text{Na}_i^3}{K_{iNa}^3} = C_T \frac{\gamma_o}{\gamma_i \delta_o + \gamma_o \delta_i} \frac{\text{Na}_i^3}{K_{iNa}^3}. \end{aligned}$$

Similar expressions can be derived for $\text{Ca}C_o$, $\text{CaNa}C_o$, Na_2C_o , and Na_3C_o .

2.3. EFFECT OF MEMBRANE POTENTIAL ON FLUXES

Since the free carriers and some carrier-cation complexes such as C , $\text{Ca}C$, Na_2C are negatively charged, the fluxes will be influenced by the membrane potential E_M .

The relationship between ion fluxes and transmembrane potential is complicated, and depends on the specific membrane model used. However, in conditions in which the voltage is changing so slowly that capacitative current is negligible and the electrical field inside the membrane is everywhere constant, the standard flux- E_M relationship applies. Hence the influx J_{in} and efflux J_{out} due to the concentration S_i , S_o , and E_M , are

$$\begin{aligned} J_{out} &= P_s S_i \psi_i, \\ J_{in} &= P_s S_o \psi_o, \\ \psi_i &= \frac{\xi e^{-\xi}}{1 - e^{-\xi}}, \\ \psi_o &= \frac{\xi}{1 - e^{-\xi}}, \\ \xi &= ZFE_M / RT, \end{aligned}$$

where P_s is the transport rate, Z is the valence, F is the Faraday number, R is the gas constant, and T is the absolute temperature.

The expressions for carrier complex are used to write the unidirectional flux of calcium and sodium. For example, the calcium efflux is

$$\begin{aligned} J_{iCa} &= P_{Ca} C_a C_i \psi_i + P'_{Ca} \text{CaNa}C_i, \\ &= C_T \left(\frac{P_{Ca} \psi_i}{K_{iCa}} + \frac{P'_{Ca} \text{Na}_i}{K_{iCa}''} \right) \frac{C_{a_i} \gamma_o}{\gamma_i \delta_o + \gamma_o \delta_i}. \end{aligned}$$

The sodium efflux is

$$\begin{aligned} J_{ioNa} &= P_{Na} Na_2 C_i \psi_i + P'_{Na} Na_3 C_i \\ &= C_T \left(\frac{P_{Na} \psi_i}{K_{iNa}} + \frac{P'_{Na} Na_i}{K''_{iNa}} \right) \frac{Na_i^2 \gamma_o}{\gamma_i \delta_o + \gamma_o \delta_i}. \end{aligned}$$

Similar expressions can also be written for the calcium and sodium influx.

2.4. UNIDIRECTIONAL FLUX EQUATIONS

The general Ca and Na unidirectional flux can be expressed as

$$J_{kjCa} = \frac{1}{\lambda} C_T \gamma_j Ca_k \left(\frac{P_{Ca} \psi_k}{K_{kCa}} + \frac{P'_{Ca} Na_k}{K''_{kCa}} \right), \quad (8)$$

$$\begin{aligned} J_{kjNa} &= \frac{1}{\lambda} C_T \gamma_j Na_k^2 \left(\frac{P_{Na} \psi_k}{K_{kNa}} + \frac{P'_{Na} Na_k}{K''_{kNa}} \right), \\ \gamma_j &= P_c \psi_j^3 + Na_j^2 \left(\frac{P_{Na} \psi_j}{K_{jNa}} + \frac{P'_{Na} Na_j}{K''_{jNa}} \right) + Ca_j \left(\frac{P_{Ca} \psi_j}{K_{jCa}} + \frac{P'_{Ca} Na_j}{K''_{jCa}} \right), \quad (9) \\ \delta_j &= 1 + \frac{Ca_j}{K_{jCa}} + \frac{Ca_j Na_j}{K''_{jCa}} + \frac{Na_j^2}{K_{jNa}} + \frac{Na_j^3}{K''_{jNa}}, \\ \lambda &= \gamma_o \delta_i + \gamma_i \delta_o, \quad kj=oi, io \quad j=o, i. \end{aligned}$$

Equations (8) and (9) describe the unidirectional fluxes as functions of either external or internal ionic concentrations, such as Ca_o , Na_o , Ca_i and Na_i . For example, to express the Na efflux as a function of Na_o , Eq. (9) becomes

$$J_{ioNa} = \frac{C_T \phi_{iNa} Na_i^2}{\gamma_i} \frac{\gamma_o}{\delta_o + \gamma_o \delta_i / \gamma_i}.$$

When an alkali metal ion or dextrose (AM_o) is to substitute for Na_o , so that $AM_o + Na_o =$

Na_o , then Na_o^2 / K_{oNa} is replaced by

$$(AM_o / K_{AM}^{1/2} + Na_o / K_{oNa}^{1/2})^2,$$

and γ_o

$$\begin{aligned} &= P_{Na} \psi_o [(1 + K_{Na} Na_o)(\bar{K}_{AM} + \bar{K}_{Na} Na_o)^2 + K'_{Ca} (1 + K_{Ca} Na_o)], \quad \delta_o + \gamma_o \delta_i / \gamma_i = 1 + K_{oNa} (K_{Na,io} \\ &+ K''_{Na,io} Na_o)(\bar{K}_{AM} + \bar{K}_{Na} Na_o)^2 \\ &+ (K_{Ca,io} \\ &+ K''_{Ca,io} Na_o) Ca_o. \end{aligned}$$

The sodium efflux J_{ioNa} is given by

$$J_{ioNa} = \frac{J_{iNa} P_{Na} \psi_o \left[(1 + K_{Na} Na_o)(\bar{K}_{AM} + \bar{K}_{Na} Na_o)^2 + K'_{Ca} (1 + K_{Ca} Na_o) \right]}{1 + K_{oNa} (K_{Na,io} + K''_{Na,io} Na_o)(\bar{K}_{AM} + \bar{K}_{Na} Na_o)^2 + (K_{Ca,io} + K''_{Ca,io} Na_o) Ca_o}, \quad (10)$$

where

$$\begin{aligned}
K_{Na} &= P'_{Na} K_{oNa} / P_{Na} \psi_o K''_{oNa}, \\
K_{Ca} &= P'_{Ca} K_{oCa} / P_{Ca} \psi_o K''_{oCa}, \\
K'_{Ca} &= P_{Ca} C_{a_o} / P_{Na} K_{oCa}, \\
\bar{K}_{AM} &= Na_{oM} / K_{AM}^{1/2}, \\
\bar{K}_{Na} &= \frac{1}{K_{oNa}^{1/2} - K_{AM}^{1/2}}, \\
J_{i,Na} &= C_T \phi_{iNa} Na_i^2 / \gamma_i.
\end{aligned}$$

From Eq. (10) the sodium efflux can be modified further as

$$J_{iNa} = \frac{J_{Na} \left[(1 + K_{Na} Na_o)(K'_0 + K'_1 Na_o)^2 + (1 + K_{Ca} Na_o) \right]}{1 + (K'_2 + K'_3 Na_o)(K'_0 + K'_1 Na_o)^2 + K'_4 Na_o}, \quad (11)$$

where

$$\begin{aligned}
J_{Na} &= \frac{J_{iNa} P_{Na} \psi_o K'_{Ca}}{1 + K_{Ca,io} C_{a_o}}, \\
K'_0 &= \bar{K}_{AM} / \sqrt{K'_{Ca}}, \\
K'_1 &= \bar{K}_{Na} / \sqrt{K'_{Ca}}, \\
K'_2 &= \frac{K_{oNa} K_{Na,io} K'_{Ca}}{1 + K_{Ca,io} C_{a_o}}, \\
K'_3 &= \frac{K_{oNa} K'_{Na,io} K'_{Ca}}{1 + K_{Ca,io} C_{a_o}}, \\
K'_4 &= \frac{K'_{Ca,io} C_{a_o}}{1 + K_{Ca,io} C_{a_o}}.
\end{aligned}$$

3. METHOD OF FITTING EXPERIMENTAL FLUX DATA

The flux data used in this paper were obtained from published studies. Unidirectional flux equations (8) and (9) were used to fit the experimental flux data by adjusting the numerical constants (the K 's). Usually, the published curves were magnified and then copied onto graph paper. Suitable units were then calculated. Errors were unavoidable during extraction of the data; however, the centers of the data points were used to minimize error.

The extracted data points were stored on disk (Xerox Sigma 5) and could be graphed and displayed on a terminal scope, Tektronix 4010.

The Tektronix terminal was coupled to a parameter entry device. This device permitted entry of parameters into a digital simulation program in a manner similar to parameter entry on an analogue computer. Eight potentiometers, each with a two digit identifier, were provided.

To compute the unidirectional flux, the appropriate program was recalled from the terminal. Estimated values for the K 's were entered through the potentiometers. The result was graphed and displayed along with the flux data points.

The calculated flux curve could be altered by adjusting the potentiometers. Influences of individual parameters on the calculated flux could be inspected visually. The adjustment of the parameters was terminated when the calculated flux curve passed through, or came very

close to, as many experimental data points as possible. The RMS error was not calculated because of limited availability of the data.

4. TEST OF THE MODEL AGAINST PUBLISHED DATA

The kinetic expressions defining the unidirectional flux, Eqs. (8) and (9), were fitted to data from:

1. experiments on calcium efflux and influx in guinea pig atrium,
2. calcium efflux from perfused beating rat heart,
3. sodium and calcium efflux and influx of squid axon.

Since the present model contains quite a few parameters (transport rates, binding constants, etc.), no attempt was made to supply the numerical values for these parameters. Instead, the unidirectional flux was expressed as functions of the ionic concentration by which the flux varied [Eqs. (8), (9)]. If, for example, the Ca efflux expression was fitted to experimental data as a function of Na_o , then the parameters such as P_{Ca} , P_{Na} , P'_{Ca} , P'_{Na} , Na_i , Ca_i , Ca_o , and the binding constants were grouped together and considered as “lumped” parameters. Only these lumped parameters were adjusted to give a satisfactory fit.

Coupling between inward and outward ion transport required that the mobility of the free carrier be much less than that of the combined forms; for tight coupling, then, the carrier should cross the membrane only when complexed with cation. Therefore, in all fittings of experimental data, $P_c\psi_i^3$ and $P_c\psi_o^3$ were considered insignificant and were ignored.

4.1. Ca EFFLUX IN GUINEA PIG AURICLE

Reuter and Seitz [24] found the tracer Ca efflux from guinea pig atrial tissue was increased by raising Ca_o and Na_o . When the reciprocal of the measured Ca efflux ($1/J_{\text{ioCa}}$) was plotted against $1/\text{Ca}_o$, the data appeared to be described by a straight line of the form

$$\frac{1}{J_{\text{ioCa}}} = a + b \frac{1}{\text{Ca}_o}. \quad (12)$$

The reciprocal of the Ca efflux equation [Eq. (8)] as a function of Na_o and Ca_o is

$$\frac{1}{J_{\text{ioCa}}} = \frac{1}{J_{\text{i,Ca}}} \frac{(K_{\text{Ca,io}} + K''_{\text{Ca,io}} \text{Na}) \text{Ca}_o + [1 + (K_{\text{Na,io}} + K''_{\text{Na,io}} \text{Na}_o) \text{Na}^2]}{\phi_{o,\text{Na}} \text{Na}_o^2 + \phi_{o,\text{Ca}} \text{Ca}_o}. \quad (13)$$

For Eq. (13) to be expressed in the same form as Eq. (12), the contribution of $\phi_{o,\text{Na}} \text{Na}^2$ must be insignificant or negligible when compared to $\phi_{o,\text{Ca}} \text{Ca}_o$. Hence

$$a = \frac{K_{\text{Ca,io}} + K''_{\text{Ca,io}} \text{Na}_o}{J_{\text{i,Ca}} (P_{\text{Ca}} \psi_o / K_{o\text{Ca}} + P'_{\text{Ca}} \text{Na}_o / K''_{o\text{Ca}})},$$

$$b = \frac{1 + (K_{\text{Na,io}} + K''_{\text{Na,io}} \text{Na}_o) \text{Na}_o^2}{J_{\text{i,Ca}} (P_{\text{Ca}} \psi_o / K_{o\text{Ca}} + P'_{\text{Ca}} \text{Na}_o / K''_{o\text{Ca}})}.$$

To have a good fit,

$$a = \frac{1+0.1483\text{Na}_o}{2+0.0450\text{Na}_o},$$

$$b = \frac{1+(0.1122 \times 10^{-2} + 1.5243 \times 10^{-6}\text{Na}_o)\text{Na}_o^2}{2+0.0450\text{Na}_o}.$$

The double reciprocal plot of Eq. (13) and the data of Reuter and Seitz are shown in Fig. 2. Note that as both the intercept a and the slope b of the previous linear equation are functions of Na_o , the theoretical lines do not intersect at a common point on the $1/J_{i\text{Ca}}$ axis.

Reuter and Seitz found that $J_{i\text{Ca}}$ was almost linearly related to $\text{Ca}_o/\text{Na}_o^2$ in a semilog plot and concluded that 2Na exchange with 1Ca. However, the semilog plot tends to suppress high $J_{i\text{Ca}}$ while amplifying low $J_{i\text{Ca}}$ values. If indeed, 2Na do exchange for 1Ca, Eq. (13) will be reduced to

$$\frac{1}{J_{i\text{Ca}}} = \frac{1}{J_{i\text{Ca}}} \left(\frac{K_{\text{Ca},io}}{P_{\text{Ca}}\psi_o/K_{o\text{Ca}}} + \frac{1+K_{\text{Na},io}\text{Na}_o^2}{P_{\text{Ca}}\psi_o/K_{o\text{Ca}}} \frac{1}{\text{Ca}_o} \right). \quad (14)$$

The intercept $K_{\text{Ca},io}/J_{i\text{Ca}}P_{\text{Ca}}\psi_o/K_{o\text{Ca}}$ then becomes a constant; all the lines in Fig. 2 would have the same intercept. However, data indicate that the intercepts vary with Na_o . Furthermore, a plot of $1/J_{i\text{Ca}}$ against $\text{Na}_o^2/\text{Ca}_o$ indicates a curvilinear relationship (Fig. 3) instead of a straight line, implying that the exchange of 2Na with 1Ca may not be valid.

4.2. Ca INFLUX IN GUINEA PIG ATRIUM

It has been shown [16] that the Ca influx ($J_{o\text{Ca}}$) was influenced by both Na_o and Na_i . $J_{o\text{Ca}}$ was increased by increasing Na_i or by decreasing Na_o . To describe the influx data by the present scheme, Eq. (8) is expressed as function of Na_i and Na_o :

$$J_{o\text{Ca}} = K \frac{K_0 + K_1\text{Na}_i^2 + K_2\text{Na}_i^3}{1 + K_3\text{Na}_i^2 + K_4\text{Na}_i^3},$$

$$K_0 = P_{\text{Ca}}\text{Ca}_i\psi_i/K_{i\text{Ca}},$$

$$a = 1 + \text{Ca}_iK_{\text{Ca},oi},$$

$$K = \frac{C_T\phi_{o,\text{Ca}}\text{Ca}_o}{(\phi_{o,\text{Na}}\text{Na}_o^2 + \phi_{o,\text{Ca}}\text{Ca}_o)a}, \quad (15)$$

$$K_1 = P_{\text{Na}}\psi_i/K_{i\text{Na}},$$

$$K_2 = P'_{\text{Na}}/K''_{i\text{Na}},$$

$$K_3 = K_{\text{Na},oi}/a,$$

$$K_4 = K''_{\text{Na},oi}/a.$$

Terms in $\text{Na}_i/K''_{i\text{Ca}}$ were neglected by assuming a large value for $K''_{i\text{Ca}}$. The data of Glitsch et al. [16] can be fitted satisfactorily (Fig. 4) by using the values for the K 's found in Table 1.

Since P_{Ca} , P_{Na} , P'_{Na} , ψ_i , Ca_i , $K_{i\text{Ca}}$, $K_{i\text{Na}}$, and $K''_{i\text{Na}}$ have been considered as constants, K_0 , K_1 , and K_2 do not vary with Na_o . However, K_3 , and K_4 are functions of Na_o , and change when Na_o is increased. At $\text{Na}_o = 11 \text{ mM}$, the influx data can be simply fitted by a straight line.

According to Eq. (15), in order to have $J_{i\text{Ca}}$ linearly related to Na_i^2 , then K_2 , K_3 , and K_4 should be zero. However, this is not realistic, because these K 's have definite values.

4.3. Ca EFFLUX IN RAT HEARTS

The Ca^{2+} washout data of Sidell and Bassingthwaighte [26] were obtained from isolated, beating, Tyrode-perfused rat hearts. The fractional escape rate (FER) was the rate of emergence of tracer in the efflux (CPM/min), divided by the residual tracer content (CPM)

in the organ at that time. It was found to be influenced by Na_o . The FER was lowered when Na_o was reduced. We used the Ca efflux equation Eq. (8) to describe the FER. Since the contribution of the terms involving $K''_{o\text{Na}}$ and $K''_{o\text{Ca}}$ was insignificant, this equation can be reduced to a simple form:

$$\begin{aligned} J_{\text{Ca},o} &= J_{\text{Ca},o} \frac{1 + (\text{Na}_o/K_1)^2}{1 + (\text{Na}_o/K_2)^2}, \\ \left(\frac{1}{K_1}\right)^2 &= \frac{P_{\text{Ca}} K_{o\text{Ca}}}{P_{\text{Ca}} K_{o\text{Na}} \text{Ca}_o}, \\ \left(\frac{1}{K_2}\right)^2 &= \frac{K_{\text{Na},io}}{1 + \text{Ca}_o K_{\text{Ca},io}}, \\ J_{\text{Ca},o} &= \frac{J_{\text{Ca},o} P_{\text{Ca}} \psi_{o\text{Ca}}}{K_{o\text{Ca}} (1 + K_{\text{Ca},io} \text{Ca}_o)}. \end{aligned} \quad (16)$$

$J_{\text{Ca},o}$ is the Ca-dependent Ca efflux at zero Na_o .

By setting $K_1 = 37.29 \text{ mM}$, $K_2 = 57.74 \text{ mM}$, and $J_{\text{Ca},o} = 0.45$, the FER data can be described as in Fig. 5.

4.4. EFFLUX AND INFLUX IN SQUID AXON

Experiments on squid axons, and to some extent on crab nerve, have provided evidence to support the idea of transmembrane sodium-calcium exchange [1–12]. Ample kinetic data clarifying the exchange mechanism has been provided by the works of Baker et al. and Blaustein et al. Our study would be incomplete without analyzing their data. Therefore, in this section, the model is further tested by fitting the flux data on squid axon.

Ca Influx and Na Efflux as a Function of Na_o —Baker et al. [2] demonstrated that when external Na was replaced by Li or dextrose, the Ca influx increased in the presence of 10^{-5} M ouabain. As Na_o was replaced by Li, the Ca influx increased uniformly. With dextrose as a substitute, influx increased to a peak while Na_o was reduced to about 100 mM . Further reduction of Na_o caused a fall in Ca influx. These influx data can be quantitatively described by the Ca influx equation, Eq. (8) expressed as a function of Na_o :

$$\begin{aligned} J_{\text{Ca},o} &= J_{\text{Ca},o} \frac{1 + K_1 \text{Na}_o}{(1 + K_3 \text{Na}_o^2)(1 + K_2 \text{Na}_o)}, \\ K_1 &= \frac{P_{\text{Ca}} K_{o\text{Ca}}}{P_{\text{Ca}} \psi_{o\text{Ca}} K_{o\text{Ca}}}, \\ K_2 &= \frac{K_{\text{Ca},io} \text{Ca}_o}{1 + K_{\text{Ca},io} \text{Ca}_o}, \\ K_3 &= \frac{K_{\text{Na},io}}{1 + K_{\text{Ca},io} \text{Ca}_o}, \\ J_{\text{Ca},o} &= \frac{C_T P_{\text{Ca}} \psi_{o\text{Ca}} \text{Ca}_o}{K_{o\text{Ca}} (1 + K_{\text{Ca},io} \text{Ca}_o)}. \end{aligned} \quad (17)$$

The above equation has been derived by assuming that

$$\frac{K_{\text{Na},io}}{K''_{\text{Na},io}} = \frac{1 + K_{\text{Ca},io} \text{Ca}_o}{K''_{\text{Ca},io} \text{Ca}_o}.$$

$J_{\text{Ca},o}$ is the Ca-dependent Ca influx at $\text{Na}_o = 0$.

It is not known how Li and dextrose affect the binding constants differently. But the Ca influx data can be satisfactorily fitted by setting $K_1 = K_2$ or $K_1 = 0$ when Li replaces Na_o , and $K_1 = K_2$ when dextrose replaces Na_o . Hence

$$J_{\text{Ca},o}(\text{Li}) = \frac{J_{\text{Ca},o}}{1 + K_3 \text{Na}_o^2}, \quad (18)$$

$$J_{\text{Ca},o}(\text{dextrose}) = J_{\text{Ca},o} \frac{1 + K_1 \text{Na}_o}{(1 + K_2 \text{Na}_o)(1 + K_3 \text{Na}_o^2)}.$$

Numerical values of $J_{\text{Ca},o}$ and the K 's used to fit the influx data of Baker et al. (Fig. 6) are found in Table 2.

Baker et al. [2] also showed that the sodium efflux behaved similarly to the calcium influx: the sodium efflux into Li sea water increased as Na was replaced, but peaked at $\text{Na}_o = 110 \text{ mM}$ in dextrose sea water. Equation (11) is used to quantify the efflux data:

$$J_{\text{Na}} = \frac{J_{\text{Na}} \left[(1 + K_{\text{Na}} \text{Na}_o)(K'_0 + K'_1 \text{Na}_o)^2 + (1 + K_{\text{Ca}} \text{Na}_o) \right]}{1 + (K'_2 + K'_3 \text{Na}_o)(K'_0 + K'_1 \text{Na}_o)^2 + K'_4 \text{Na}_o}. \quad (19)$$

Table 3 lists K and J_{Na} values to fit this data satisfactorily.

Figure 7 depicts the data of Baker et al. [2] and the theoretical curves.

Figures 6 and 7 indicate that in the presence of ouabain both Ca influx and Na efflux are increased in a similar manner by the removal of Na_o . These results strongly suggest a coupling between an inward movement of calcium and an outward movement of sodium.

Effect of Na_i on Ca Efflux—One argument Blaustein et al. used to support the theory of an exchange ratio of $3\text{Na}:1\text{Ca}$ is the graphical relation between Na_i and the increase or decrease of calcium efflux; the curve is considerably flatter than anticipated if 2Na^+ ions displace 1Ca^{2+} . In this section the data of Blaustein et al. are fitted by the following equation:

$$J_{\text{Ca},o} = J_{\text{Ca},o} \frac{1 + K_0 \text{Na}_i}{1 + (K_1 + K_2 \text{Na}_i) \text{Na}_i^2 + K_3 \text{Na}_i},$$

$$K = 1 + \text{Ca}_i K_{\text{Ca},io},$$

$$J_{\text{Ca},o} = C_T P_{\text{Ca}} \text{Ca}_i \psi_i / K_{\text{Ca}} K,$$

$$K_0 = P'_{\text{Ca}} K_{\text{Ca}} / P_{\text{Ca}} K''_{\text{Ca}}, \quad (20)$$

$$K_1 = K''_{\text{Na},oi} / K,$$

$$K_2 = K''_{\text{Na},oi} / K,$$

$$K_3 = \text{Ca}_i / K''_{\text{Ca},oi} K.$$

In Fig. 11 of Blaustein et al. [9] the Ca efflux is about $4 \text{ P mole/cm}^2\text{sec}$ at $\text{Na}_i = 50 \text{ mM}$; an increase of $2 \text{ P mole/cm}^2\text{sec}$ can be extrapolated from their Fig. 12. The K 's and $J_{\text{Ca},o}$ are

$$J_{\text{Ca},o} = 6 \text{ P mole/cm}^2\text{sec},$$

$$K_0 = 1.322 \text{ mM}^{-1},$$

$$K_1 = 1.224 \times 10^{-2} \text{ mM}^{-2},$$

$$K_2 = 0.356 \times 10^{-4} \text{ mM}^{-3},$$

$$K_3 = 1.300 \text{ mM}^{-1}.$$

Based on an exchange ratio of 2Na^+ to 1Ca^{2+} , K''_{Na} and K''_{Ca} become infinite and the above equation is reduced to

$$J_{ioCa} = \frac{J_{Ca,o}}{1 + K_1 Na_i^2}. \quad (21)$$

To satisfy the requirement that $J_{ioCa} = 4$ P mole/cm²sec at $Na_i = 50$ mM and $= 6$ P mole/cm²sec at $Na_i = 0$, then K_1 must equal 2×10^{-4} mM⁻². Equation (21) based on a ratio of $2Na^+$ to $1Ca^{++}$ (dashed line, Fig. 8) does not describe the data satisfactorily.

Ouabain-Insensitive Na Efflux—Baker et al. [2] showed that with 10^{-5} M ouabain to inhibit the Na-K pump, the Na efflux was influenced by Ca_o . With dextrose replacing Na_o , the maximal efflux occurred at $Na_o = 100$ mM, but declined as Na_o was increased. Equation (19) can be used to describe the data. However, Eq. (10) is used to show the effect of Ca_o :

$$J_{ioNa} = J'_{iNa} \frac{(1 + K_{Na} Na_o)(\bar{K}_{AM} + \bar{K}_{Na} Na_o)^2 + K'_{Ca}(1 + K_{Ca} Na_o)}{1 + (K_1 + K_2 Na_o)(\bar{K}_{AM} + \bar{K}_{Na} Na_o)^2 + K_3(1 + K_4 Na_o)}, \quad (22)$$

where

$$\begin{aligned} J'_{iNa} &= J_{iNa} P_{Na} \psi_o, \\ K_1 &= K_{oNa} K_{Na,io}, & K_2 &= K_{oNa} K''_{Na,io}, \\ K_3 &= K_{Ca,io} Ca_o, & K_4 &= K''_{Ca,io} / K_{Ca,io}. \end{aligned}$$

The other K 's were defined in Eq. (10).

Since K'_{Ca} and K_3 are functions of Ca_o , these two parameters become zero at $Ca_o = 0$; the other K 's should remain unchanged.

Table 4 presents the K values used to fit the Na efflux data depicted in Fig. 9.

Effect of Cations on Na Efflux in the Presence of Ouabain—When different cations, such as K, Li, Na, or choline, replace dextrose, they have various effects on sodium efflux. Baker et al. [2] found that choline had no activating effect, but Li, Na, K all increased the sodium efflux at low concentrations. Maximal sodium efflux occurred at about 100 mM but declined at higher concentrations of Li and Na. K, however, activated sodium efflux even at higher concentrations. Equation (11) is used to describe these efflux data; AM_o replaces Na_o to represent the concentration of other cations. Therefore,

$$J_{ioNa} = \frac{J_{Na} \left[(1 + K_M AM_o)(K'_0 + K'_1 AM_o)^2 + (1 + K_{Ca} AM_o) \right]}{1 + (K'_2 + K'_3 AM_o)(K'_0 + K'_1 AM_o)^2 + K'_4 AM_o}. \quad (23)$$

If K_{oNa} and K''_{oNa} are replaced by K_{oM} and K''_{oM} , and P_{Na} , P_{Na} by P_M , P'_M , then all the K 's are defined exactly like those in Eq. (11). Taking J_{Na} as the relative fraction of ²²Na lost per minute at $AM_o = 0$, the data shown in Fig. 10 are fitted by the numerical parameters found in Table 5.

The reason for $K'_0 = 0$ is that at $AM_o = 0$:

$$J_{ioNa} = J_{Na} \frac{K_0'^2 + 1}{1 + K_1' K_0'^2}.$$

Since the relative sodium efflux is 1 at $AM_o = 0$, then K_0' has to be zero in order that $J_{Na} = 1$.

The Effect of Na_o on Ca Efflux—The Ca efflux of a squid axon is promoted by increasing Na_o . However, the data show that at $Na_o = 0$, the Ca efflux is negligibly small. According to Eq. (8), which relates Ca efflux to Na_o , the term $\phi_{o, Ca}$ must be small and can be considered as zero. Thus Eq. (8) can be simplified to

$$\begin{aligned} J_{ioCa} &= J_{max} \frac{1 + K_0/Na_o}{1 + K_1/Na_o + (K_2/Na_o)^2 + (K_3/Na_o)^3}, \\ K_0 &= \frac{P_{Na} \psi_o}{P_{Na}} K_{Na_2}, \\ K_1 &= (1 + P_{Na} \psi_o \delta_i / \gamma_i) \frac{K_{Na_2}}{1 + P_{Na} \delta_i / \gamma_i}, \\ K_2 &= \frac{(1 + P_{Ca} \delta_i / \gamma_i) Ca_o}{K_{oCa} (1 + P_{Na} \delta_i / \gamma_i)} K_{Na_2} K_{oNa}, \\ K_3 &= \frac{1 + \frac{K_{oCa}}{1 + P_{Ca} \delta_i / \gamma_i}}{1 + P_{Ca} \delta_i / \gamma_i} K_{Na_2} K_{oNa}, \\ J_{max} &= \frac{J_{iCa} P_{Na}}{1 + P_{Na} \delta_i / \gamma_i}, \end{aligned} \quad (24)$$

where K_{oNa} and K_{Na_2} are the binding constants of the following reactions:

When it is assumed that ATP only increases the affinity of carriers to external Na and internal Ca in the divalent sites, only K_{oNa} and K_{Ca} are reduced. K_0 will remain unchanged with or without ATP. The term δ_i / γ_i , which contains K_{Ca} , will be affected, but can be assumed to change negligibly. Thus only K_2 and K_3 are significantly affected by a decrease of K_{oNa} .

Figure 11 shows that the Ca efflux data of Blaustein [12] can be fitted satisfactorily with the numerical constants in Table 6. Note that only K_{oNa} in K_2 and K_3 is reduced by a factor of $(36/90)^3$. The idea that a single carrier mechanism is used to extrude Ca in exchange for Na or for another Ca was supported by his experiment (Fig. 5) [12]. His Ca efflux data are shown in Table 7.

Experimental data [9] have shown that Na-dependent Ca efflux is fully activated by a solution containing only half the normal concentrations of Na_o (212.5 mM Na_o), while the Ca-dependent Ca efflux is activated by a solution containing only 212.5 mM Li.

Based on the above observations, Blaustein [12] argued that the Ca efflux in a solution containing 212.5 mM Na_o + 212.5 mM Li should be approximately equal to the sum of the Na-dependent and Ca-dependent (Li-stimulated) Ca efflux if Ca is extruded by an independent mechanism. The data show that Ca efflux into Na + Li is almost the same as into 425 mM Na_o and much lower than the efflux into 425 mM Li. Therefore, Na- and Ca-dependent Ca efflux are not independent, and Na-Ca and Ca-Ca exchange are mediated by a single carrier system.

Since the present model implies simultaneous exchange, the data of Blaustein [12] can be simulated with the following assumptions:

1. The binding constants between Li and Ca are not the same as between Na and Ca if the solution only contains Li.
2. In a solution containing both Li and Na the Li-Ca and Na-Ca binding constants are the same.

Hence the equation for the Ca efflux as a function of Na_o and Li can be written as

$$J_{ioCa} = J_{\max} \frac{1 + K_0/M_o}{1 + K_1 M_o + (K_2/M_o)^2 + (K_3/M_o)^3}, \quad (25)$$

where

$$M_o = \frac{Li}{\sqrt{K_{Li}}} + \frac{Na_o}{\sqrt{K_{Na}}}.$$

By setting $J_{\max} = 1.82$ P mole/cm²sec, $K_0 = 4.5$, $K_1 = 3.0$, $K_2 = 75/\sqrt{2}$, $K_3 = 75$, and $K_{Li} = 1$ when $Li = 425$ mM, $Na_o = 0$, and $1/\sqrt{K_{Li}} = 1/\sqrt{K_{Na}} = 0.145$ in the Li + Na_o mixture, the calculated ⁴⁵Ca efflux (listed in the bottom line of Table 7), is comparable to the observed flux data.

The calculation has assumed that in a solution containing both Li and Na, Li behaves like Na: at equal concentrations, the binding constants are the same. It is not known whether the binding of Li to carrier is affected by the Li concentration.

Ca Efflux and Internal Ca—We have demonstrated that Na_o and Ca_o activate Ca efflux. The Ca efflux equation (8) also shows that both Ca_i and Na_i promote Ca efflux. This is the case in guinea pig auricle [24] and in squid axon [10]. A simplification procedure similar to that applied to Eq. (12) can reduce Eq. (8) to the following form:

$$J_{ioCa} = \frac{Ca_i}{b + a Ca_i},$$

$$a = \frac{K_{Ca,oi} + K''_{Ca,oi} Na_i}{J_{o,Ca} (P_{Ca} \psi_i / K_{iCa} + P'_{Ca} Na_i / K''_{iCa})}, \quad (26)$$

$$b = \frac{1 + (K_{Na,oi} + K''_{Na,oi} Na_i) Na_i^2}{J_{o,Ca} (P_{Ca} \psi_i / K_{iCa} + P'_{Ca} Na_i / K''_{iCa})}.$$

The Ca efflux data are quantitatively described by the solid line [Fig. 12(a)] calculated from Eq. (26) with the following numerical values for a and b :

$$a = \frac{0.0927 + 0.4154 \times 10^{-2} Na_i}{0.1859 + 0.1497 \times 10^{-2} Na_i},$$

$$b = \frac{1 + 0.2116 \times 10^{-2} Na_i^2}{0.1859 + 0.1497 \times 10^{-2} Na_i},$$

where b is in μM / (P mole/cm²sec) and a is in P mole/cm²sec; $K''_{Na,oi}$ is found insignificant. The broken lines are calculated from the equation of Blaustein and associates:

$$J_{ioCa} = \frac{1.7 \text{ P mole/cm}^2\text{sec}}{1 + (8/Ca_i)(1 + Na_i/30)^2}. \quad (27)$$

The reciprocals of both Eqs. (26) and (27) relate $1/J_{ioCa}$ linearly to $1/Ca_i$ so that

$$\frac{1}{J_{ioCa}} = a + \frac{b}{Ca_i}.$$

The main difference between the two is that in Eq. (26) a is a function of Na_i while in Eq. (27) it is a constant ($1/1.7$). When an exchange of 3Na with 1Ca is assumed, a should be dependent on Na_i . Therefore Eq. (26) gives a better fit to the efflux data.

The calcium efflux, promoted by internal calcium (Ca_i) and internal sodium (Na_i), is increased by ATP [12]. With the assumption that ATP increases K_{oNa} and K_{iCa} , and that δ_i/γ_i is not affected significantly, the ATP-fueled Ca efflux data can be best described by a tenfold reduction of K_{iCa} and an increase $\times 1.5$ in $K''_{Ca,oi}$. The other parameters such as K''_{iCa} , $K''_{Ca,oi}$ are assumed unaltered.

Table 8 lists numerical values of a and b used to fit the efflux data at $Na_i = 5 \text{ mM}$ [Fig. 12(b)], according to Eq. (26).

4.5. Ca EFFLUX AND MEMBRANE POTENTIAL

An exchange ratio of 3Na: 1Ca indicates that the unidirectional flux is influenced by the membrane potential because of an unequal movement of charges in opposite directions across the membrane. Mullins and Brinley [20] and Blaustein et al. [9] observed that depolarization inhibits Ca efflux. In our model, the flux of the carrier-cation complexes Na_2C , CaC (which are negatively charged) depends on the membrane potential.

When the ionic concentrations remain unchanged, the Ca efflux can be expressed as a function of the membrane potential E_M :

$$J_{ioCa} = \frac{P_{Ca} C_T Ca_i \left(\psi_i + \frac{P'_{Ca} K_{iCa} Na_i}{P_{Ca} K_{iCa}} \right) \left(\psi_o + \frac{b_o}{a_o} \right)}{\delta_i K_{iCa} \left[\left(\psi_o + \frac{b_o}{a_o} \right) + \left(\psi_i + \frac{b_i}{a_i} \right) \frac{a_i \delta_o}{a_o \delta_i} \right]}, \quad (28)$$

where

$$\begin{aligned} a_j &= \frac{P_{Na} Na_j^2}{K_{jNa}} + \frac{P_{Ca} Ca_j}{K_{jCa}}, \\ b_j &= \frac{P'_{Na} Na_j^3}{K_{jNa}} + \frac{P'_{Ca} Ca_j Na_j}{K_{jCa}}, \\ j &= o, i, \\ \psi_i &= \frac{\xi e^{-\xi}}{1 - e^{-\xi}}, \\ \psi_o &= \frac{\xi}{1 - e^{-\xi}}, \\ \xi &= FE_m/RT. \end{aligned}$$

Likewise, the Ca influx J_{oCa} is

$$J_{oCa} = \frac{P_{Ca} C_T Ca_o \left(\psi_o + \frac{P'_{Ca} K_{oCa} Na_o}{P_{Ca} K'_{oCa}} \right) \left(\frac{a_i}{a_o \psi_i} + \frac{b_i}{a_o} \right)}{\delta_i K_{oCa} \left[\left(\psi_o + \frac{b_o}{a_o} \right) + \left(\frac{a_i \psi_i}{a_o} + \frac{b_i}{a_o} \right) \frac{\delta_o}{\delta_i} \right]}. \quad (29)$$

We assumed that $P'_{Ca} = P_{Ca}$, $P'_{Na} = P_{Na} \gg P_C$, and K''_{oCa} and K''_{iCa} were very large, so the ratio of influx (J_{oCa}) to efflux (J_{iCa}) is

$$\frac{J_{oCa}}{J_{iCa}} = \frac{a_i}{a_o} \frac{Ca_o}{Ca_i} \frac{K_{iCa}}{K_{oCa}} \frac{\psi_o(\psi_i + b_i/a_i)}{\psi_i(\psi_o + b_o/a_o)}. \quad (30)$$

We also assumed that $b_i/a_i > \psi_i$ and $b_o/a_o > \psi_o$; the flux ratio then becomes

$$\begin{aligned} \frac{J_{oCa}}{J_{iCa}} &\approx \frac{b_i}{b_o} \frac{Ca_o}{Ca_i} \frac{K_{iCa}}{K_{oCa}} \frac{\psi_o}{\psi_i} \\ &\approx \left(\frac{Na_i}{Na_o} \right)^3 \frac{Ca_o}{Ca_i} \frac{K_{iCa}}{K_{oCa}} \frac{K''_{oNa}}{K''_{iNa}} e^{\xi}. \end{aligned} \quad (31)$$

Based on the same assumptions, Eq. (28) can be simplified to

$$J_{iCa} \approx F(Na_o, Ca_o, Ca_i, Na_i) \psi_i,$$

where

$$F = \frac{(P_{Ca} C_T Ca_i / \delta_i K_{iCa})(b_o/a_o)}{b_o/a_o + b_i \delta_i / a_o \delta_i}$$

is a function of the internal and external Na and Ca concentrations. When these ionic concentrations are kept constant, the Ca efflux is simply a function of membrane potential:

$$J_{iCa} = F \frac{\xi e^{-\xi}}{1 - e^{-\xi}}. \quad (32)$$

Figure 13 shows the normalized Ca efflux, influx, and influx/efflux, as well as the Ca efflux data on squid axon [9]. Ca efflux is inhibited and Ca influx is promoted as the membrane potential becomes less negative. Note, however, that the calculated fluxes assume that the ionic concentrations are unchanged. According to Eq. (31) and Fig. 13, the Ca influx is greater than the Ca efflux at positive potentials. Ca_i therefore increases, promoting Ca efflux, and decreasing the ratio of influx to efflux. At negative potentials, as Ca efflux is increased, Ca_i is reduced: this, in turn, inhibits Ca efflux. The actual Ca efflux at negative potentials should be less than the calculated ones, as is confirmed by the data of Blaustein et al.

At zero potential, the normalized influx/efflux is unity. In fact, this ratio [Eq. (31)] depends on $(Na_i/Na_o)^3 (Ca_o/Ca_i)$ as well as on the binding constants. Assuming that the binding constants are equal and the ionic concentrations are $Na_i = 4 \text{ mM}$, $Na_o = 147 \text{ mM}$, $Ca_o = 1.8 \text{ mM}$, and $Ca_i = 10^{-4} \text{ mM}$, we have (Ca influx) = (Ca efflux) at $E_M = 13 \text{ mV}$. Doubling Ca_o shifts the reversal potential to 4 mV; halving Na_o reduces this potential to -13 mV .

5. DISCUSSION

Recently, Mullins [21] proposed a Na-Ca transport model which is, in essence, the first quantitative scheme for Na-Ca exchange. Various similarities and differences exist between his model and our model.

Both models assume carriers on both sides of the membrane and utilize the electrochemical gradient of Na^+ for the source of free energy. The influence of membrane potential on fluxes is taken into account.

While the present scheme proposes an exchange of 3Na^+ for 1Ca^{++} , Mullins's model requires 4Na^+ to a carrier before a Ca binding site is induced. In our model, the fully or partially loaded carrier-ion complexes are mobile and diffusible across the membrane; thus the unidirectional flux is contributed by these carrier-ion complexes. In Mullins's model, however, Na and Ca binding sites must be fully occupied to be mobile. The partially loaded carriers—those with less than 4Na^+ —cannot carry Ca^{++} . Subsequently, influx and efflux are due to fully loaded carriers only.

Mullins's model explains the activating and the inhibiting effect of Na on the Ca transport reaction from a theoretical approach; ours attempts to explain the exchange mechanism by fitting curves to the experimental data.

Our study shows that an exchange of 3Na to 1Ca apparently describes the flux data quite satisfactorily. Since the coupling ratio is different from 2: 1, the exchange is no longer electroneutral, but electrogenic. Blaustein et al. [9] and Mullins and Brinley [20] have demonstrated the inhibition of Ca efflux in squid axons by the membrane potential. Our model also shows the effect of the membrane potential on Ca efflux, with data similar to that of Blaustein et al., although the numerical values of the parameters are empirical. Although the preliminary experiment by Watson and Winegard [28] suggested an influence of transmembrane potential on Ca efflux in skeletal muscle, its effect on ionic fluxes in cardiac muscle has not been demonstrated. Recently, Jundt et al. [17] studied Na-activated Ca efflux from guinea pig auricles in hypertonic solution in which KCl and NaCl could be changed over a wide range. They concluded that the Na-activated Ca efflux did not depend on membrane potential. However, these investigators only demonstrated the percentage increase of Na-activated Ca efflux at -76 mV and -12 mV respectively; they did not compare the Ca-efflux at -76 mV with that at -12 mV . Their efflux data (their Fig. 9), in fact, suggested a decrease of Ca efflux at steady state when KCl was increased from 5.4 to 105.4 mM .

Throughout our study, we determined only the “lumped” parameters. Ideally, such parameters as P , P' , K , K'' , C_T should be evaluated, as they are physiologically significant. Unfortunately, the available data do not make this possible. Our model contains 8 binding constants and 4 transport rate coefficients. To determine the absolute values of these unknowns, 12 equations are required. Because it is difficult to estimate Ca_i and Na_i , most of the flux equations are expressed in terms of 6 or 7 “lumped” parameters which have to be adjusted to fit the data. It might seem that with 6 or 7 unknowns to be manipulated any curve could be produced. However, the parameters were not selected to fit just one set of data; they were determined to fit a series of data sets. For example, in the Ca-Ca exchange [Fig. 12(a)], the K 's were adjusted to fit the Ca flux data at $\text{Na}_i = 100\text{ mM}$ concentration. Then the same set of K 's were used to fit the other Ca flux data at $\text{Na}_i = 25$ and 5 mM . Most of the K 's were determined under such strict constraints. The process of curve fitting in our study is not the usual polynomial fit.

Could another set of numerical K 's fit the data as well? The answer depends on the number of data to be fitted. If there are ample data, the "lumped" K 's are unlikely to change. When there are only a few points spread over a wide range, another set of numerical K 's could probably produce a good fit. However, 3Na appear to be required to have a good fit, according to our results.

Recent studies [5,14,20] have indicated that ATP promotes Ca extrusion. The role of ATP has not been incorporated into the present model. Within the present scheme, the energy required for the extrusion of Ca against a large Ca gradient has to be derived from the Na and electrochemical gradient. The simplified steady state equation

$$\frac{Ca_i}{Ca_o} = \left(\frac{Na_i}{Na_o} \right)^3 \exp \left(\frac{FE_M}{RT} \right)$$

gives a ratio of $Ca_i / Ca_o = 10^{-4}$ in squid axon [9]. A similar Ca gradient can be calculated for cardiac muscle. However, analysis of the flux data indicates that in most cases the binding constants do not have equal values. Equation (31) therefore should be used to calculate the Ca gradient. In squid axon, at $E_M = -60$ mV, $Na_i / Na_o = 4 / 14$, a Ca gradient of 10^{-4} can be obtained if $K_{iCa} K''_{oNa} / K_{oCa} K''_{iNa} = 0.5$. For cardiac muscle, when $E_M = -85$ mV and $Na_i / Na_o = 8 / 137$, the same Ca gradient (10^{-4}) can be maintained when the ratio of the binding constants is about 2. This calculation suggests that when the binding constants are asymmetrical, the Na and voltage gradients can provide an adequate supply of energy to power Ca efflux.

Our model considers the transport rate coefficients and the binding constants as constants, unaffected by the internal or external milieu. With these assumptions Eq. (26) satisfactorily describes the Ca efflux as function of Ca_i below $160 \mu M$. However, at $Ca_i = 560 \mu M$, Blaustein and Russell [10] observed an efflux of 5.37 P mole/cm² set, while Eqs. (26) and (27) only predict an efflux of 1.67 P mole/cm²sec. They attributed the discrepancy to the possible increase of $K_{Ca,i}$ and maximal efflux, and this raised the possibility that the ionic environment influences the kinetic parameters of the Ca carriers.

If the binding constants K_{iCa} and K''_{iCa} in Eq. (26) are increased 3.5 times as Ca_i is raised from 160 to $560 \mu M$, then a is not changed and b is reduced; this will result in only a slight increment of efflux. When $P_{Ca} \psi_i$ is increased 3.5 times and K_{iCa} and K''_{iCa} remain unchanged, both $K_{Ca,oi}$ and $K''_{Ca,oi}$ will increase slightly due to the small value of P_{Ca} , and $J_{oCa} P_{Ca} \psi_i / K_{iCa}$ will increase 3.5 times. Thus, at $Na_i = 5$ mM, $a = 0.1864$, and $b = 1.6000$, the predicted Ca efflux is 5.28 P mole/cm²sec, which is comparable to the measured efflux (5.37). Although ψ_i is influenced by the membrane potential E_M , which may be altered by high Ca_i , a 3.5-fold increase of Ca_i does not increase ψ_i proportionately. To account for the large increase, P_{Ca} has to be increased. If this is true, the transport ion rate coefficients may be a function of ionic concentration.

6. CONCLUSION

An exchange ratio of 3Na: 1Ca appears to satisfactorily describe the influx data on excitable tissue. We emphasize that our model is not a unique one; some other scheme may well produce similar results. Also, the good fit to the flux data does not necessarily validate the model; it merely expresses the data in a quantitative manner.

Glossary

NOMENCLATURE

C	Concentration of unoccupied carrier.
Na	Sodium concentration, molar.
C_T	Total concentration of carrier.
CaC , $CaNaC$, Na_2C , Na_3C	Ca-carrier-complex concentration.
J_j, Ca	$C_T Ca_j \phi_j, Ca / \gamma_j$
J_j, Na	$C_T Na_j^2 \phi_{jNa} / \gamma_j$, $j=i, o$.
K_{iCa} , K_{oCa} , K_{iNa} , K_{oNa}	K 's for divalent binding (e.g. $K_{iCa} = Ca_i C_i / CaC_i$).
K''_{iCa} , K''_{oCa} , K''_{iNa} , K''_{oNa}	K 's for binding 1Na to carrier – divalent-ion complex e.g. $K''_{iNa} = Na_i Na_2 C_i / Na_3 C_i$).
K_S, lm	Concentration-weighted factor = $(1 + P_S \psi_m \delta_l / \gamma_l) / K_{mS}$.
$K''_{S,lm}$	$= (1 + P_S \delta_l / \gamma_l) / K''_{mS}$, concentration-weighted factor, $S=Na, Ca$, $l=i, o$, $m=0, i$; e.g. $K_{Na,io} = \frac{1 + P_{Na} \psi_o \delta_i / \gamma_i}{K''_{oNa}}$, $K''_{Ca,io} = \frac{1 + P_{Ca} \delta_i / \gamma_i}{K''_{oCa}}$.
Na_o, M	Maximal external Na_o .
P_C , P_{Ca} , P_{Na} , P'_{Ca} , P'_{Na}	Carrier transport rate constants for C , CaC , Na_2C , $CaNaC$, Na_3C , in sec^{-1} .
γ_j	Concentration-dependent rate, constant for all forms of carrier, in sec e.g. $\gamma_o = P_C + P_{Ca} Ca_o / K_{oCa} + P'_{Ca} Ca_o Na_o / K_0 + P_{Na} Na_o^2 / K_{oNa} + P'_{Na} Na_o^3 / K''_{oNa}$.
δ_j	Concentration-distributed factor, dimensionless.
ϕ_j, Na	$P_{Na} \psi_j / K_{jNa} + P'_{Na} Na_j / K''_{jNa}$.
ϕ_j, Ca	$P_{Ca} \psi_j / K_{jCa} + P'_{Ca} Na_j / K''_{jCa}$.

SUBSCRIPTS

i	Inner surface of membrane
o	Outer surface of membrane
io	In-to-out flux

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FIG. 1.

Schematic representation of proposed model for sodium-calcium exchange. The free carriers, triply charged, have two binding sites. One site can bind a single Na ; the second site can bind 2Na or 1Ca . Other alkali metal ions such as Li^+ , K^+ can compete with Na in the second site. M represents a single Na ion with which alkali metal ions do not compete.

FIG. 2. Description of data of Reuter and Seitz [24] by the solid lines calculated from Eq. (13). Two broken lines are predicted $1/J_{ioCa}$ at $Na_0 = 0, 200 \text{ mM}$.

FIG. 3. i/J_{i0Ca} , versus $[Na_0]^2/Ca_0$ plot of the data of Reuter and Seitz. The line was drawn by eye. The curvilinear line indicates the dependence of the slope on Na_0 . With an exchange of 2Na : 1Ca, the slope should be constant.

FIG. 4.

The Ca influx data on guinea pig auricle [Eq. (14)] at $\text{Na}_o = 149 \text{ mM}$ (○) and 11 mM (●) as a function of Na_i^2 . The solid lines are computed from Eq. (15). Data are from Glitsch et al. [16].

FIG. 5. Comparison of model with data on ^{45}Ca efflux from beating rat hearts. Solid line is computed from Eq. (16). Data are from Sidell and Bassingthwaighte [26].

FIG. 6.
Ca influx graphed as function of Na_o in Li-Na mixture and dextrose-Na mixture. Data are taken from Baker et al. [2].

FIG. 7. The Na efflux in a Li-Na mixture (broken line, ●) and a dextrose-Na mixture (solid line, ■), graphed as a function of Na_o. Data are from Baker et al. [2].

FIG. 8.

The increase and decrease in Ca efflux relative to the efflux at $Na_i = 50$ mM. An exchange of 3Na: 1Ca [Eq. (20), solid line] describes data (●) of Blaustein et al. [9] better than a ratio of 2Na: 1Ca [Eq. (21), broken line].

FIG. 9. The Ouabain-insensitive Na efflux at $\text{Ca}_0 = 11 \text{ mM}$ (●) and $\text{Ca}_0 = 0$ (▲) as a function of Na_0 . Data are from Baker et al. [2]; solid lines are computed from Eq. (22).

FIG. 10.

The effect of cation concentration on sodium efflux in $10^{-5} M$ Ouabain. AM_0^+ is the cation concentration in mM. ●, Na; ○, Li; □ K; ■, choline. Data are from Baker et al. [2].

FIG. 11. Effect of ATP on Ca efflux of squid axon, expressed as percentage of maximal, is depicted as function of Na_0 . Data are from Blaustein [12].

FIG. 12.
 (a) Effect of internal ionized Ca concentration on Na-dependent Ca efflux at Na_i = 5 mM (●), Na_i = 25 mM (Δ), and Na_i = 100 mM (○). Solid lines are calculated from Eq. (26), and broken lines from Eq. (27). Data are from Blaustein and Russell [10]. (b) Effect of ATP on Ca efflux at Na_i = 5 mM. Data are from Blaustein [12].

FIG. 13. Simulated effect of membrane potential on Ca efflux [Eq. (32)] and Ca influx [Eq. (32)]. Ca efflux data (Δ , $\circ$, $\bullet$) on squid axon (Fig. 5, Blaustein et al. [9]) are superimposed for comparison.

TABLE 1*K* Values to Fit Ca Influx Data in Guinea Pig Atrium

Value	Na _o concentration	
	149 mM	11 mM
$K [(mM/kg)/(10 \text{ min})]$	0.300	0.450
$K_0 [(mM/kg)/(10 \text{ min})]$	1.000	1.000
$K_1 [10^{-3} mM^{-2}/(10 \text{ min})]$	0.967	0.967
$K_2 [10^{-5} mM^{-3}/(10 \text{ min})]$	1.333	1.333
$K_3 [10^{-3} mM^{-2}]$	0.115	0.104
$K_4 [10^{-5} mM^{-3}]$	0.350	0.322

TABLE 2 $J_{Ca, o}$ and K Values to Fit Ca Influx Data in Squid Axon

Media replacing Na_o	$J_{Ca, o}$ (P mole/ cm^2sec)	K_1 (mM^{-1})	K_2 (mM^{-1})	K_3 (mM^{-2})
Li	47.14			$15.5/(460)^2$
Dextrose	21.45	1/460	1/230	$15.5/(460)^2$

TABLE 3 J_{Na} and K Values to Fit Na Efflux Data in Squid Axon

Value	Media replacing Na_o	
	Li	Dextrose
J_{Na} (P mole/cm ² sec)	19.00	4.46
K_{Na} (mM ⁻¹)	5.80×10^{-3}	5.65×10^{-2}
K'_0	0.695×10^{-2}	0.287×10^{-3}
K'_1 (mM ⁻¹)	0.662×10^{-3}	1.324×10^{-2}
K_{Ca} (mM ⁻¹)	8.70×10^{-4}	5.65×10^{-2}
K'_2	63.332	15.889
K'_3 (mM ⁻¹)	0.275	0.276
K'_4 (mM ⁻¹)	$\approx 0.$	1.73×10^{-6}

TABLE 4

K Values to Fit Ouabain-Insensitive Na Efflux Data

Value	Ca_o concentration	
	11 mM	0 mM
$J'_{i,\text{Na}}$	0.294×10^{-4}	0.294×10^{-4}
$K_{\text{Na}} (10^{-3} \text{ mM}^{-1})$	2.113	2.113
$\bar{K}_{\text{AM}}$	1.870	1.870
$\bar{K}_{\text{Na}} (10^{-3} \text{ mM}^{-1})$	9.565	9.565
K'_{Ca}	11.750	0.000
$K_{\text{Ca}} (10^{-3} \text{ mM}^{-1})$	8.522	8.522
K_1	$\approx 0.$	$\approx 0.$
$K_2 (10^{-3} \text{ mM}^{-1})$	0.322	0.322
K_3	0.605	0.000
$K_4 (10^{-3} \text{ mM}^{-1})$	0.087	0.087

TABLE 5 J_{Na} and K Values to Fit Na Efflux Data in Presence of Ouabain

Values	Cation replacing dextrose			
	Choline	Na	Li	Potassium
J_{Na}	1.0	1.0	1.0	1.0
$K_M(10^{-2} \text{ mM}^{-1})$	0.1736	0.0539	0.8076	1.6883
K'_0	0.0	0.0	0.0	0.0
$K'_1(10^{-2} \text{ mM}^{-1})$	0.6705	0.9682	0.7457	0.5261
$K_{\text{Ca}}(\text{mM}^{-1})$	0.0	0.1012	0.0521	0.1063
K'_2	0.9715	0.1717	0.3146	0.4719
$K'_3(10^{-2} \text{ mM}^{-1})$	0.1308	0.4690	0.4592	0.4568
$K'_4(10^{-1} \text{ mM}^{-1})$	0.0	0.3764	0.1248	0.2413

TABLE 6*K* Values to Fit Ca Efflux Data Influenced by Na_o

Value (mM)	ATP	No ATP
K_0	4.67	4.67
K_1	3.0	3.0
K_2	$22.8/\sqrt{2}$	$90/\sqrt{2}$
K_3	36.0	90.0

TABLE 7

Ca Efflux Data ^a						
Solution						
	Na	Li	Li/choline	Na/Li	Na	
Conc. (mM):	425	425	212.5/212.5	212.5/212.5	425	425
⁴⁵ Ca efflux						
(P mole/cm ² sec):						
Observed	≈0.5	≈1.8	≈1.8	≈0.7	≈0.6	
Calculated	0.54	1.8	1.66	0.54	0.54	0.54

^aBlaustein et al. [12].

TABLE 8Values for a and b to Fit Ca Efflux Data at $\text{Na}_i = 5 \text{ mM}$

Value	ATP	No ATP
a [P mole/cm ² sec]	0.513	0.587
b [$\mu\text{M}/(\text{P mole/cm}^2\text{sec})$]	0.563	5.440