Translocation Mechanism of Na-Ca Exchange in Single Cardiac Cells of Guinea Pig

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ABSTRACT We have studied in single cardiac ventricular cells of guinea pig the ionic translocation mechanism of the electrogenic Na-Ca exchange, i.e., whether Na and Ca ions countercross the membrane simultaneously or consecutively with “ping pong” kinetics. The dose–response relation between the external Ca concentrations ([Ca]o) and the current density of the outward Na-Ca exchange current were measured at three different intracellular Na concentrations ([Na]i) in the absence of external Na. Nonlinear regression curves of the dose–response relation obtained by computer revealed Michaelis-Menten type hyperbola from which the [Ca]o giving a half-maximal response (apparent K MyClass) and the apparent maximum current magnitude (I[max]) were estimated at each [Na]i. As [Na]i increased, the K MyClass increased progressively and the value of K MyClass/I[max] tended to decrease. These results are consistent with the simultaneous mechanism. The K MyClass/I[max] values, however, were small and close to each other, so it was not possible to completely preclude a consecutive mechanism.

INTRODUCTION

In the cardiac myocyte, the electrogenic Na-Ca exchange is a major sarcolemmal calcium efflux system (Mullins, 1981; Langer, 1982; Noble, 1986; Carafoli, 1987). Whether the mechanism of the exchange is simultaneous or consecutive has not yet been resolved (Johnson and Kootsey, 1985; Läuger, 1987; Hilgemann, 1988). In the simultaneous exchange scheme, both Na and Ca bind to the exchanger and then the translocation takes place. In the consecutive scheme, Na binds to the exchanger and is translocated, and then Ca binds and is translocated, so that Na and Ca cross the membrane consecutively with “ping pong” kinetics. The method of examining these different mechanisms has been well established in enzyme kinetics (Segel, 1975) and has been applied to various other membrane transport systems (Läuger, 1987; Hilgemann, 1988). Recently the membrane current generated by the Na-Ca ex-
change has been isolated by loading Na and Ca on the opposite side of the membrane while blocking almost all other ionic currents in single cardiac myocytes using patch clamp and intracellular dialysis techniques (Hume and Uehara, 1986; Kimura et al., 1986; Mechmann and Pott, 1986). This method allows detailed kinetic studies on the Na-Ca exchange (Kimura et al., 1987; Gadsby et al., 1988; Beuckelmann and Wier, 1989; Miura and Kimura, 1989). In this study we examined the transport mechanism of the Na-Ca exchange according to the enzyme kinetics by measuring the outward exchange current under the condition that inward exchange current could not flow.

METHODS

The methods of cell preparation, whole-cell voltage clamp, and the intracellular dialysis were previously described in detail (Miura and Kimura, 1989). In brief, single ventricular cells were isolated from guinea pig heart by perfusing collagenase (8 mg/50 ml; Yakult, Tokyo, Japan) in Ca-free Tyrode solution on the Langendorff apparatus. The patch pipette for the whole-cell voltage clamp had a tip diameter of 4-5 μm (resistance of 1-2 MΩ).

After establishing a GΩ seal with normal Tyrode solution in the glass pipette, the test internal solution containing a high concentration of BAPTA (Ca-chelating agent) and various concentrations of Na was introduced into the pipette via a thin polyethylene tubing with negative pressure. The patch membrane was then ruptured to perform whole-cell clamp. We waited at least 5 min to equilibrate the cell interior with the internal solution before giving test clamp pulses. The current–voltage (I-V) relation was obtained by ramp pulses of ±100 mV from the holding potential of −10 mV at the rate of 0.4 V/s, which was recorded on-line with a personal computer (NEC PC9801-VM). The descending portion of the ramp was used for display (Miura and Kimura, 1989).

Solutions

The compositions of external and internal solutions are described in Tables I and II, respectively. The concentration of free Ca was calculated by the equations of Fabiato and Fabiato (1979) with the correction of Tsien and Rink (1980) using the binding constants of BAPTA (Tsien, 1980). All the experiments were performed at ~36°C.

Data

The numerical data are shown as the mean ± SE unless otherwise stated.

Theory

The two types of transport kinetics, consecutive and simultaneous, show characteristic rate equations that can be distinguished graphically when plotted by a Hanes-Woolf linear plot as shown in Fig. 1, according to the following theory:

(a) The consecutive model is called the “ping pong bi bi” system in Segel’s enzyme kinetics (1975; p. 606), and the scheme of one-way reaction, i.e., Na–Ca exchange in the presence of Na only inside and Ca outside, is as follows:

\[
\begin{align*}
\text{Na}_i & \quad \text{Na}_o \\
\downarrow & \quad \downarrow \\
E & \quad 
\text{ENa} \leftrightarrow \text{ENa}_o \\
& \quad F \\
\text{Ca}_o & \quad \text{Ca}_i \\
\uparrow & \quad \uparrow \\
F \text{Ca}_o & \leftrightarrow F \text{Ca}_i \\
& \quad E
\end{align*}
\]
### TABLE I

**Composition of External Solution**

<table>
<thead>
<tr>
<th></th>
<th>mM</th>
</tr>
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<tbody>
<tr>
<td>Li</td>
<td>150</td>
</tr>
<tr>
<td>Mg</td>
<td>0.5</td>
</tr>
<tr>
<td>Cl</td>
<td>150</td>
</tr>
<tr>
<td>EGTA</td>
<td>5</td>
</tr>
<tr>
<td>Free Ca</td>
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</tr>
<tr>
<td>HEPES</td>
<td>10</td>
</tr>
<tr>
<td>Ouabain</td>
<td>0.02</td>
</tr>
<tr>
<td>Verapamil</td>
<td>0.002</td>
</tr>
</tbody>
</table>

pH 7.4.

where E and F are forms of the exchanger that have a high affinity to Na and Ca, respectively. E\(\text{Na}\) is an exchanger-Na complex and E\(\text{Ca}\) is an exchanger-Ca complex (subscript o or i indicates external or internal, respectively). The steady-state velocity equation is as follows (Segel, 1975; p. 608, IX-142):

\[
\frac{i}{I_{\text{max}}} = \frac{[\text{Ca}]_o[\text{Na}]^n_i}{K_{\text{m}}N_{\text{a}}[\text{Ca}]_o + K_{\text{m}}C_{\text{a}}[\text{Na}]_i^n + [\text{Ca}]_o[\text{Na}]_i^n}
\]

where \(K_{\text{m}}C_{\text{a}}\) is the Michaelis constant for [Ca] \(o\) or the concentration of [Ca] \(o\) that gives half-maximal response at saturating [Na] \(o\); \(K_{\text{m}}N_{\text{a}}\) is the Michaelis constant for [Na] \(i\), at saturating [Ca] \(o\); \(I_{\text{max}}\) is the maximum current magnitude, and n is the Hill coefficient for [Na] \(i\) (Segel, 1975; p. 360). The Hill coefficient of 2 is best fitted for internal Na (Miura and Kimura, 1989). \(I_{\text{max}}\) values and \(K_{\text{m}}\) values for Na and Ca are constant. In the enzyme kinetics v

### TABLE II

**Composition of Internal Solutions**

<table>
<thead>
<tr>
<th></th>
<th>150 mM Na</th>
<th>30 mM Na</th>
<th>20 mM Na</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na</td>
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</tr>
<tr>
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<td>Tris</td>
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<td>5</td>
<td>5</td>
</tr>
<tr>
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<td>45</td>
</tr>
<tr>
<td>Cl</td>
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<td>30</td>
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</tr>
<tr>
<td>ATP</td>
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<td>10</td>
<td>10</td>
</tr>
<tr>
<td>CrP</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>BAPTA</td>
<td>30</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Ca</td>
<td>14.5</td>
<td>14.5</td>
<td>14.5</td>
</tr>
<tr>
<td>HEPES</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

pCa 7, pH 7.4.
and $V_{\text{max}}$ are usually used to express the velocity of the reaction and the maximum velocity, respectively. In our experiment, however, the membrane current indicates the amount of the reaction, so $i$ is used instead of $v$ and $I_{\text{max}}$ instead of $V_{\text{max}}$. Eq. 1 can be transformed as the following equation for a linear plot between $[\text{Ca}]_o/i$ and $i$ (Hanes-Woolf plot):

$$\frac{[\text{Ca}]_o}{i} = \frac{(K_m\text{Na}_i)^n}{I_{\text{max}}} + \frac{(\text{Na})^n}{I_{\text{max}}} + \frac{1}{I_{\text{max}}} [\text{Ca}]_o.$$  

This equation is in the form of $y = a + bx$, where $y = [\text{Ca}]_o/i$ and $x = [\text{Ca}]_o$. In this equation, the value of the y intercept $(K_m\text{Ca}_o/I_{\text{max}})$ is constant regardless of $[\text{Na}]$, while the slope decreases as $[\text{Na}]$ increases and the absolute value of the x intercept, which is called the “apparent” $K_m\text{Ca}_o$, increases as $[\text{Na}]$ increases. The graph of this model is shown in Fig. 1.

(b) The simultaneous (also called sequential) exchange mechanism is that both Na and Ca initially bind the exchanger and then are translocated in the opposite direction at the same time. Among the various types of simultaneous reactions (Segel, 1975), two representative types are a “random bi bi” system and an “ordered bi bi” system. In the “random bi bi” system the order of binding and dissociation of Na and Ca to the exchanger molecule are at random, while in the “ordered bi bi” system Na and Ca bind and dissociate in a definite order. The rate equations for the two types of simultaneous models are similar, however, and their graphical patterns are identical. Therefore we use the rate equation of an “ordered bi bi” system in the following scheme to represent the simultaneous models:

1. Consecutive

2. Simultaneous

![Figure 1](image-url)  

**Figure 1.** Expected patterns of a Hanes-Woolf plot of the consecutive model and three distinct simultaneous models. $K_m$ represents the Michaelis constant and $K$ represents the dissociation constant. See text for explanation.

For $[\text{Na}]_i-[\text{Ca}]_o$ exchange, the steady-state velocity equation is as follows (Segel, 1975; p. 564, IX-89):

$$\frac{i}{I_{\text{max}}} = \frac{[\text{Ca}]_o[\text{Na}]^n}{K_{\text{Ca}}(K_m\text{Na}_i)^n + (K_m\text{Na}_i)^n[\text{Ca}]_o + K_{\text{Ca}}\text{Ca}_o[\text{Na}]^n + [\text{Ca}]_o[\text{Na}]^n}.$$  

$E\text{CaNa}$ is the exchanger-Ca-Na complex. For $[\text{Na}]_i-[\text{Ca}]_o$ exchange, the steady-state velocity equation is as follows (Segel, 1975; p. 564, IX-89):
$K_{Ca_o}$ is the dissociation constant of $[Ca]_o$ and the other symbols are the same as in Eq. 1. Arranging Eq. 3 for a linear Hanes-Woolf plot gives the following:

$$\frac{[Ca]_o}{i} = \frac{(K_{mNa})^nK_{Ca_o}}{([Na])^n + K_{mNa} + 1} \frac{([Na])^n}{i_{max}} [Ca]_o.$$

This equation is also the form of $y = a + bx$, where $y = [Ca]_o/i$ and $x = [Ca]_o$. In contrast to Eq. 2, the value of $a$, or the $y$ intercept, is a function of $[Na]$. Thus, if $[Na]$ increases, the value of the $y$ intercept decreases. The slope is the same as that in Eq. 2.

The plot of Eq. 4 further varies according to the ratio of $K_{mCa_o}$ and $K_{Ca_o}$ (see Fig. 1): (a) If $K_{mCa_o} > K_{Ca_o}$, the lines obtained at different $[Na]$ will intersect at a point on the left of the $y$ axis and the absolute value of the $x$ intercept ($K'_{Ca_o}$) will increase as $[Na]$ increases. (b) If $K_{mCa_o} = K_{Ca_o}$, the $K'_{Ca_o}$ will be constant regardless of $[Na]$. (c) If $K_{mCa_o} < K_{Ca_o}$, the $K'_{Ca_o}$ will decrease as $[Na]$ increases.

Thus, in order to distinguish the Na-Ca exchange reaction among the above four models, the values of the apparent $K_{mCa_o}$ ($K'_{Ca_o}$) and the apparent $I_{max}$ ($I'_{max}$) are examined at various $[Na]$, in the following experiment.

**RESULTS**

It has been suggested that it is advantageous to study the translocation mechanism under “zero trans” conditions (Läuger, 1987). Therefore, we set the ionic conditions to induce only an outward exchange current by loading $[Na]$ only inside and $[Ca]$ outside. $[Ca]$ cannot be eliminated because it is necessary for the exchange to operate (Baker, 1972; Baker and McNaughton, 1976; Allen and Baker, 1985; Kimura et al., 1986), so a minimum amount of 100 nM free $[Ca]$ was added in all the internal solutions. Although it has been reported that the Na-Ca exchange also operates as a Ca-Ca exchange (Blaustein, 1977; Philipson and Nishimoto, 1981; Ledvora and Hegyvary, 1983; Slaughter et al., 1983) and that $[Ca]$ may inhibit the binding of $[Na]$ (Reeves and Sutko, 1983), we assumed that the fraction of Ca-Ca exchange was small enough and that $[Ca]$ would not compete effectively with $[Na]$ at 100 nM $[Ca]$ and 20 mM or higher $[Na]$. These assumptions are based on the reports that Ca-Ca exchange is inhibited in the presence of a high concentration of Na (Ledvora and Hegyvary, 1983; Slaughter et al., 1983) and that the $K_i$ (inhibition constant) for $[Na]$ is ~10 mM at $[Ca] > 10 \mu M$ in vesicle studies (Reeves and Sutko, 1983).

As shown in Fig. 2, top, a brief superfusion of various $[Ca]$ at a holding potential of −10 mV induced an outward exchange current in the presence of 150 mM $[Na]$. The control external solution is Ca free (0, 1, or 5 mM EGTA and no added Ca) and the test solution contains either 0.1, 0.25, 0.5, 1, 2, or 3 mM free Ca. The $I-V$ relations obtained by the ramp pulses of ±100 mV from the holding potential before and during the $[Ca]$ superfusion are shown in Fig. 2, A−D (bottom). As the current becomes larger, it decays after the peak, probably due to accumulation of Ca and/or depletion of Na under the membrane. To avoid this artifact, the external solution was changed as quickly as possible and the $I-V$ relation was measured at the earliest possible phase of the peak. The net exchange current was obtained as the difference between each two traces, and the representative $I-V$ curves are superimposed in Fig.
Figure 2. Top, Chart records of the outward shift of the holding current at -10 mM by a brief application of [Ca]o from 0.1 to 1 mM as indicated below by the bar of each trace. [Na]i is 150 mM. The vertical lines indicate the current in response to ramp pulses of ±100 mV to -10 mV. Bottom, I-V relations are taken at the corresponding labels in the inset. The I-V curves of a, c, e, and g are the control and b, d, f, and h are during [Ca]o superfusion. The data in A-D are taken from different cells. The capacitance of the cells was 181, 121, 172, and 184 pF in A-D, respectively.

3 C. Since one concentration of Ca was tested in each cell in most of the experiments, the current density was calculated by dividing the current magnitude by the capacitance of the cell to compare the current density in different cells.

The I-V curves of the exchange current were obtained not only at 150 mM [Na]i but also at 20 and 30 mM [Na]i and the representative difference currents are superimposed at each [Na]i in Fig. 3. As [Na]i increased, the magnitude of the current density increased. The current density was measured at four different potentials, i.e., +50, +30, 0, and -50 mV, and was plotted against [Ca]o to obtain the dose-response relations at all [Na]i.

Fig. 4 shows the four sets of dose-response relations at the different potentials. The curves are drawn by nonlinear regression analysis (Marquardt method) using a SAS computer program (SAS Inc. Ltd., Cary, NC). The data are fitted well by

Figure 3. The superimposed difference currents between the control and the peak response during [Ca]o superfusion. A, 20 mM [Na]i; B, 30 mM [Na]i; C, 150 mM [Na]i (obtained from Fig. 2). The current magnitudes are expressed as the current density. The average capacitance of the cells is 156 ± 39 pF (n = 35) at 20 mM [Na]i, 153 ± 49 pF (n = 28) at 30 mM [Na]i, and 129 ± 37 pF (n = 28) at 150 mM. The current is larger as [Na]i and [Ca]o are higher. The vertical dotted lines indicate the potentials where the current magnitudes are measured for the dose-response relation.
hyperbola, indicating that the reaction is Michaelis-Menten type. Sakoda and Hiromi (1976) demonstrated that in order to estimate $K_m$ and $V_{max}$ values of the Michaelis-Menten type reaction, the direct nonlinear fitting method with iterations is better than any linear plot analysis. Therefore, we made a computer program according to

![Graphs A, B, C, D showing dose-response curves between $[Ca]_o$ (abscissa) and the magnitude of the current density (ordinate) measured at four different voltages as indicated in Fig. 3. The data are expressed as the mean ± SE. A, +50 mV; B, +30 mV; C, 0 mV; D, -50 mV. Solid circles indicate the currents at 150 mM $[Na]_i$, open circles 30 mM $[Na]_i$, and solid triangles 20 mM $[Na]_i$. The number of cells at each point in B–D are the same as in A. The curves were drawn by the nonlinear regression (NLIN) procedure using a SAS computer program (Marquardt method). $K_m'Ca_o$ appears to increase as $[Na]_i$ increases.]

Sakoda and Hiromi (1976) and carried out their method of inserting initial values of $K_m'Ca_o$ and $I_{max}$, which we obtained by two different linear plots with the least-squares fit, i.e., Hanes-Woolf plot ($[Ca]_o/i$ vs. $[Ca]_o$) and Lineweaver-Burk plot ($1/i$ vs. $1/[Ca]_o$). The two sets of slightly different initial values of $K_m'Ca_o$ and $I_{max}$ resulted
in an identical set of values after a number of iteration procedures in the program as described by Sakoda and Hiromi (1976). Furthermore, we also used a SAS computer program which produced (also after the iteration procedure, but without requiring the initial values) the same set of \( K_m'Ca_o \) and \( I_{\text{max}}' \) values as those obtained above by Sakoda and Hiromi's method. The results are shown in Table III.

Since the linear plots help to distinguish the different mechanisms from their characteristic patterns as shown in Fig. 1, the four sets of Hanes-Woolf linear plots of \( K_m'Ca_o / I_{\text{max}}' \) against \( K_m'Ca_o \) are made from the pairs of values in Table III (Fig. 5). At all the potentials measured, the absolute value of \( K_m'Ca_o \) increased as \([Na]_i \) increased, while the value of \( K_m'Ca_o / I_{\text{max}}' \) decreased. All the lines cross each other to the left of the y axis. The x values of the points of intersection are averaged at each potential and indicated in Table III.

**DISCUSSION**

The above results indicate that as \([Na]_i \) increases the apparent \( K_m'Ca_o \) (\( K_m'Ca_o \)) increases. The \( K_m'Ca_o \) values at 150 mV [Na] are more than twice as large as those at 20 or 30 mM [Na], at most of the potentials measured. This result clearly precludes the possibility of the two types of simultaneous models, one with \( K_m = K_d \) and the other with \( K_m < K_d \), and is consistent with both the simultaneous model with \( K_m > K_d \) and the consecutive model.

### Table III

<table>
<thead>
<tr>
<th>( E_m ) (mV)</th>
<th>[Na] (mM)</th>
<th>( K_m'Ca_o \pm SE ) (mM)</th>
<th>( I_{\text{max}}' \pm SE ) (( \mu A/\mu F ))</th>
<th>( K_m'Ca_o/I_{\text{max}}' \pm SE ) (( \mu A/\mu F ))</th>
<th>( \text{n Crossover point} \pm SE ) (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>20</td>
<td>0.17 ± 0.062</td>
<td>6.74 ± 0.58</td>
<td>0.025 ± 0.015</td>
<td>33</td>
</tr>
<tr>
<td>30</td>
<td>0.18 ± 0.055</td>
<td>9.11 ± 0.87</td>
<td>0.020 ± 0.005</td>
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<td>-0.079 ± 0.039</td>
</tr>
<tr>
<td>150</td>
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<td>0.017 ± 0.002</td>
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<tr>
<td>30</td>
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<td>0.029 ± 0.016</td>
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<tr>
<td>30</td>
<td>0.19 ± 0.058</td>
<td>8.43 ± 0.81</td>
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<td>-0.088 ± 0.026</td>
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<tr>
<td>150</td>
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<td>28.88 ± 1.41</td>
<td>0.018 ± 0.002</td>
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<tr>
<td>0</td>
<td>20</td>
<td>0.17 ± 0.059</td>
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<tr>
<td>-50</td>
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<tr>
<td>30</td>
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<td>0.051 ± 0.013</td>
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<tr>
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<td>16.21 ± 0.98</td>
<td>0.024 ± 0.006</td>
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</tbody>
</table>

\( E_m \) indicates the membrane potential, \( K_m'Ca_o \) indicates the apparent \( K_m \) of \([Ca]_o \); \( I_{\text{max}}' \) is the apparent maximum current magnitude; \( n \) is the number of experiments. The mean crossover point was obtained by averaging the x axis values of each three points of intersection. The negative sign indicates that the point of intersection lies to the left of the y axis. \( K_m'Ca_o \) and \( I_{\text{max}}' \) are expressed as the mean ± SE. \( K_m'Ca_o/I_{\text{max}}' \) was calculated from the values in the preceding columns, while the standard errors of \( K_m'Ca_o/I_{\text{max}}' \) in the parentheses were obtained by a linear regression analysis as described in the text.

The above results indicate that as \([Na]_i \) increases the apparent \( K_m'Ca_o \) (\( K_m'Ca_o \)) increases. The \( K_m'Ca_o \) values at 150 mV [Na] are more than twice as large as those at 20 or 30 mM [Na], at most of the potentials measured. This result clearly precludes the possibility of the two types of simultaneous models, one with \( K_m = K_d \) and the other with \( K_m < K_d \), and is consistent with both the simultaneous model with \( K_m > K_d \) and the consecutive model.
The value of $K_m^{Ca}/I_{\text{max}}$ tends to decrease with increasing $[Na]_i$ and all the lines plotted on the Hanes-Woolf linear plot intersect each other to the left of the $y$ axis rather than on the $y$ axis as shown in Fig. 5. This result is apparently consistent with the simultaneous mechanism with $K_m > K_d$. The value of $K_m^{Ca}/I_{\text{max}}$ at 20 mM $[Na]_i$ is approximately twice as that at 150 mM $[Na]_i$. However, these values are so small (~40 and 20 μM/μA per μF at 20 and 150 mM $[Na]_i$, respectively, at 0 mV) and the points of interactions so close to the $y$ axis (~0.1 mM at 0 mV) that these data could be within the range of variation. Since the $K_m^{Ca}/I_{\text{max}}$ values shown in Table III were calculated from the best estimates of $K_m^{Ca}$ and $I_{\text{max}}$, it was not possible to estimate the standard error. Therefore, the standard error was estimated by Hanes-Woolf linear plot ([Ca]_o/i vs. [Ca]_o) and the values are shown in the parentheses in Table III. However, standard errors obtained in this way are not small enough to indicate the significance at different $[Na]_i$ nor are they large enough to verify the insignificance. Therefore, although there is a tendency for $K_m^{Ca}/I_{\text{max}}$ to decrease as $[Na]_i$, it cannot conclude that the simultaneous model with $K_m > K_d$ is more likely than the consecutive mechanism.

Figure 5. Hanes-Woolf plot obtained by plotting the two values of $K_m^{Ca}$ and $K_m^{Ca}/I_{\text{max}}$ from Table III on the $y$ and $x$ axis, respectively. $A$, +50 mV; $B$, +30 mV; $C$, 0 mV; $D$, -50 mV. The symbols are the same as in Fig. 4. The three lines cross to the left of the $y$ axis at all the potentials measured. As $[Na]_i$ increases, the absolute value of $K_m^{Ca}$ increases and $K_m^{Ca}/I_{\text{max}}$ decreases. These results are consistent with the simultaneous mechanism.
In the present study the ionic condition is not strictly "zero trans," since 100 nM free Ca is present in the pipette solution. In addition, when the outward exchange current flows, $[Ca]_i$ could have accumulated and $[Na]_i$ depleted, which could affect the results. The possible influence of these phenomena is the following: The accumulation of $[Ca]_i$ and depletion of $[Na]_i$ could cause underestimation of the current magnitude, since $[Ca]_i$ may have inhibited $[Na]_i$ binding and a depletion in $[Na]_i$ itself decreases the outward exchange current. The level of underestimation is likely to be higher the larger the current magnitude is, in other words, at higher $[Na]_i$ and $[Ca]_o$. This suggests that the real $K_m^{Ca_o}$ and $I_{max}$ values might be larger than the presented values at higher $[Na]_i$ in Table III. Thus, in the Hanes-Woolf linear plot in Fig. 5, if there were no such artifacts the slope of the line at 150 mM $[Na]_i$ would be smaller than what is shown. As a consequence, the difference among the values of $K_m^{Ca_o}/I_{max}$ would increase, indicating an even stronger possibility of a simultaneous mechanism.

The simultaneous mechanism has been suggested by various previous workers including Baker and McNaughton (1976) and Blaustein (1977). In Blaustein's experiment of measuring $[Na]_o$-dependent $^{45}$Ca efflux, changing $[Ca]_i$ three different concentrations, i.e., -0.31, -0.5, and 2.5 μM, did not change the $K_m^{Na_o}$ of 50 mM in perfused squid axon. Philipson and Nishimoto (1982) examined $[Na]_i$-dependent $^{45}$Ca uptake in dog ventricular vesicles, and at $[Na]_i$ of 140, 56, and 28 mM the same $K_m^{Ca_o}$ of 28 μM was found. Hodgkin and Nunn (1987) also favored a simultaneous model in the salamander rod because the fractional saturation by internal Ca does not affect the external Na activation kinetics. Ledvora and Hegvarya (1983) also concluded that the mechanism was simultaneous in cardiac vesicles because the $K_m$ for $[Ca]_o$ did not depend on $[Na]_i$ and remained 30 μM. Their observation was carried out in the presence of ionic flux in both directions. All other workers have done the experiments under nominally "zero trans" conditions. The reason why others did not see the shift of $K_m$ values might be that the range of investigation was too small and/or that the range of shift of the $K_m$ value was too small to detect. Indeed, in our results the shift of $K_m^{Ca_o}$ was only from -0.2 to 0.5 mM for a 7.5-fold change of $[Na]_i$ from 20 to 150 mM. Also, if the experiment was performed without buffering Ca, micromolar amounts of Ca contamination might have distorted the nominal "zero trans" condition.

The maximum current density was strikingly large, i.e., 32 μA/μF at +50 mV at 150 mM $[Na]_i$. Even though the ionic condition was under high $[Ca]_o$ and $[Na]_i$ in the absence of counter flow of the exchange current, the value indicates a significant capacity of Ca transport by the exchanger. Using the value of 32 μA/μF, we calculated the density of exchange carriers based on estimates that the turnover number for the exchanger is 1,000/s (Cheon and Reeves, 1988). The calculated carrier density was 20 × $10^{19}$/μF or $40 \times 10^6$ per cell, assuming the capacity of a ventricular cell to be 200 pF. This value is similar to the estimated density of the Na-K pump molecule (26 × $10^6$ per cell; Bahinski et al., 1988).

The Na-Ca exchange current is voltage dependent. The voltage-dependent step in the reaction may be indicated in the kinetic values shown in Table III. The values of $K_m^{Ca_o}$ are similar among the different potentials at 20 and 30 mM $[Na]_i$ so that the
$K_m \text{Ca}_o$'s do not seem voltage dependent. The mean crossover point indicates the dissociation constant of $[\text{Ca}]_o (K\text{Ca}_o)$ if the reaction is simultaneous. The $K\text{Ca}_o$ seems voltage dependent in Table III, since the absolute value of $K\text{Ca}_o$ becomes progressively smaller at more positive potentials. The direction of the change of the $K_d$ value is consistent with the evidence that the outward exchange current is larger at more positive potentials.

Although the graphical analysis of the present data apparently favors the simultaneous mechanism, the statistical significance to preclude the possibility of the consecutive model could not be obtained. Some other approach is therefore required to confirm the result. One possibility is a product inhibition study that would not only distinguish between the consecutive and simultaneous mechanisms but also between an ordered and a random reaction, if the mechanism is simultaneous (Segel, 1975).

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