SATURATION OF THE INTERNAL SODIUM SITE OF THE SODIUM PUMP CAN DISTORT ESTIMATES OF POTASSIUM AFFINITY

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ABSTRACT The Na^+/K^+ exchange pump in cardiac Purkinje strands has been well studied with the voltage clamp and Na^+-selective microelectrodes. Models describing the observed results suggest that the pump rate can be considered proportional to $[Na^+]_i$ over the range examined and depends on external $[K^+]$ in accordance with Michaelis-Menten kinetics. Estimates of the external $[K^+]$ that achieves a half-maximal pump rate (K_m) range from 0.9 to 6.3 mM depending on the preparation and method of estimation. Here we show that much of the variability in the estimates of the K_m can be eliminated when saturation of the internal Na^+ pump site is taken into account. If the half-activation concentration for saturation of this Na^+ site is sufficiently high (>20 mM), removal of intracellular Na^+ in response to a Na^+ load will approximate first-order kinetics. Under these conditions however, Na^+ saturation will nevertheless cause large systematic errors in estimates of the K^+ dependence of pump activity.

INTRODUCTION

The activity of the Na^+/K^+ exchange pump has been extensively studied in red blood cells, squid axons, mammalian nonmyelinated nerve, molluscan neurons, and skeletal and cardiac muscle (Garay and Garrahan, 1973; Schwartz et al., 1975; Hodgkin and Keynes, 1955; Baker et al., 1969; Rang and Ritchie, 1968; Thomas, 1969, 1972; Keynes and Swan, 1959; Glitsch et al., 1978; Gadsby and Cranefield, 1979; Gadsby, 1980; Eisner and Lederer, 1980; Eisner et al., 1981). In each of these tissues the rate of Na^+ extrusion shows a saturable dependence on external $[K^+]$. Intracellular $[Na^+]$ also affects the rate of pumped Na^+ efflux. However, whether the efflux rate exhibits a saturable or nonsaturable dependence on $[Na^+]_i$ seems to depend on the preparation involved. The pumped Na^+ efflux has been found to saturate as $[Na^+]_i$ is increased in red blood cells, barnacle muscle, striated muscle, and guinea pig atrial muscle, while squid axons, molluscan neurons, and cardiac Purkinje strands show a nonsaturable first-order dependence of pump rate on $[Na^+]_i$ (Garay and Garrahan, 1973; Brinley, 1968; Keynes, 1965; Glitsch et al., 1976; Baker et al., 1969; Thomas, 1972; Gadsby, 1980; Eisner et al., 1981).

There are several reasons why an apparent linear relationship between $[Na^+]_i$ and pump activity might be observed. First, the Na^+ affinity of the internal Na^+ binding site may be lower in these preparations. Alternatively, the coupling ratio of Na^+ efflux to K^+ influx may depend on $[Na^+]_i$ (e.g., Mullins and Brinley, 1969; but see Gadsby, 1980) or, at higher $[Na^+]_i$, the measured parameter may no longer represent Na^+/K^+ pump activity (Deitmer and Ellis, 1978). Another possibility is that the actual $[Na^+]$ near the internal binding site is not the same as the measured $[Na^+]_i$. Last, if the initial interaction of Na^+ with the internal binding site was rate limiting rather than the diffusion of Na^+ (as is usually assumed), then saturation might not be seen (Schwartz et al., 1975). Here we examine the effects of the first alternative on the measured Na^+/K^+ pump rate constant and estimated K_m (the external $[K^+]$ at which the pump activity is half-maximally activated) under conditions in which Na^+/K^+ pump activity shows an apparent linear dependence on $[Na^+]_i$.

Experiments on Purkinje fibers have led to the development of quantitative models describing Na^+/K^+ pump activity. These experiments employed exposures to K^+-free solution to load Na^+ in the intracellular compartment. Upon return to K^+-containing solution, the rate constant of Na^+ removal can be obtained from either the electrogenic pump current (Gadsby, 1980; Eisner and Lederer, 1980) or decay of intracellular Na^+ activity (Deitmer and Ellis, 1978; Eisner et al., 1981). From this protocol we derive a kinetic measure of the K_m (since intracellular Na^+ is not in a steady state). Using this protocol on Purkinje fibers, the relationship between the Na^+/K^+ pump rate and $[K^+]_o$ was found to approximate a rectangular hyperbola with half-maximal saturation occurring at a $[K^+]_o$ ranging from 0.9 to 6.3 mM (Gadsby, 1980; Eisner and Lederer, 1980; Eisner et al., 1981; Deitmer and Ellis, 1978). K_m values for K^+ activation of the red blood cell Na/K pump are much less variable and fall at the low end of this range, near 1–2

mM $[K^+]_o$ (Garrahan and Glynn, 1967; Post et al., 1960; Whittam and Ager, 1964).

An alternative determination of the Na^+/K^+ pump rate can be obtained from measures of the steady-state activity of intracellular Na^+ upon long-term exposure to K^+ solutions of different concentration. From these steady state measures of a_{iNa} a value of ~1 mM for the K_m was obtained. This value for K_m was much lower than the 4 mM estimate obtained from the kinetic measure in the same series of experiments (Eisner et al., 1981).

Many assumptions are involved in estimating the K_m for K^+ in both the kinetic and steady state measures. Here we will discuss one particular assumption implicit in both kinetic and steady state estimates of K_m: the assumed lack of saturation of the intracellular Na^+ binding site. Since Na^+/K^+ pump activity experimentally appears to show a linear dependence on $[Na^+]_i$, it has been assumed that any effects of Na^+ binding site saturation must be negligible over the concentration range examined. However, we will show that this assumption has not been sufficiently tested, and that an exponential decay in response to even a large Na^+ load is not sufficient to demonstrate the absence of a saturable dependence of pumped Na^+ efflux on intracellular $[Na^+]$. The values of the apparent rate constant and K_m determined assuming nonsaturability are significantly different from their values under the same conditions but assuming saturation. Saturation of the intracellular Na^+ binding site may account for much of the variation among kinetic measurements and for the discrepancy between kinetic and steady state measurements of the K^+ dependence of Na^+/K^+ pump activation in cardiac Purkinje strands.

RESULTS

Steady State Estimates of K^+ Activation are not Severely Distorted by Saturation of the Internal Na^+ Site

In the steady state, Na^+ influx and Na^+ efflux are balanced so that the cell neither gains nor loses Na^+. The first assumption that has been made in estimating the K^+ activation curve from the intracellular Na^+ activity is that the Na^+/K^+ pump is the only available efflux route (Gadsby, 1980; Eisner et al., 1981; see Discussion). Thus

$$I_{inb} = -BI_p, \tag{1}$$

where I_{inb} is the net Na^+ influx through passive channels, I_p is the pump current, and B is the number of Na^+ ions transported per cycle divided by the difference between the number of Na^+ ions and K^+ ions transported per cycle. In the absence of a saturable internal Na^+ site

$$\frac{B\,I_p}{F\,V} = \frac{d\,[Na^+]_i}{dt} = -\,k\,[Na^+]_i, \tag{2}$$

where F is Faraday's constant, V is the intracellular volume, and k is the first-order rate constant that is presumed $[Na^+]_i$-insensitive, but dependent on the extracellular $[K^+]$.

$$k = \frac{k_{max}\,[K^+]_o}{K_m + [K^+]_o}, \tag{3}$$

where k_{max} is the apparent rate constant as $[K^+]_o \rightarrow \infty$, and K_m is the half activation concentration for K^+ on the external pump site. Combining Eqs. 1–3 gives

$$\frac{I_{inb}}{F\,V} = \frac{-B\,I_p}{F\,V} = \frac{k_{max}\,[K^+]_o}{K_m + [K^+]_o} \cdot [Na^+]_i. \tag{4}$$

A second assumption now comes into play. The background Na^+ influx is assumed independent of the external $[K^+]$ (Gadsby, 1980; Eisner et al., 1981; Daut, 1983; see Discussion). With this assumption I_p in the steady state is independent of $[K^+]_o$ so that

$$\frac{[K^+]_o}{K_m + [K^+]_o} \cdot [Na^+]_i = \text{constant}. \tag{5}$$

Table I, column A, shows the estimated values of $[Na^+]_i$ in different external $[K^+]$'s on the assumption of a K_m for $[K^+]_o$ of 1 mM and a value of $[Na^+]_i$ of 6 mM when $[K^+]_o$ is 4 mM. Fig. 1*A* demonstrates the use of a Lineweaver-Burk plot for estimating the K_m for $[K^+]_o$ from the intracellular Na^+ activity. A correct estimate of 1 mM is obtained.

Now let us consider the effects of a saturable internal Na^+ site. The rate of Na^+ extrusion is now described by

$$\frac{d\,[Na^+]_i}{d\,t} = \frac{-k_{maxx}\,[Na^+]_i}{[Na^+]_i + K_{Na}}, \tag{6}$$

where K_{Na} is the $[Na^+]_i$ at which Na^+/K^+ pump rate is half maximal, and k_{maxx} is the maximal reaction rate at a given $[K^+]_o$ when $[Na^+]_i$ approaches ∞.

Given the earlier assumptions concerning the K^+ dependence of the rate constant, the independence of the Na^+ influx from the $[K^+]_o$, and the unimportance of alternative

TABLE I
DEPENDENCE OF $[Na^+]_i$ ON K_{Na} GIVEN A TRUE VALUE OF 1 mM FOR K_m, AND $[Na^+]_i$ = 6 mM WHEN $[K^+]_o$ = 4 mM*

$[K^+]_o$	A No saturation $[Na^+]_i$	B K_{Na} = 100 $[Na^+]_i$	C K_{Na} = 40 $[Na^+]_i$	D K_{Na} = 20 $[Na^+]_i$
mM		*mM*		
0.5	14.4	15.7	18.1	24.1
1.0	9.6	10.0	10.5	11.7
2.0	7.2	7.3	7.3	7.7
4.0	6.0	6.0	6.0	6.0
8.0	5.4	5.4	5.3	5.3

*Values are calculated for no saturation of the internal Na^+ site and for half-saturation values, K_{Na} = 20 mM, 40 mM, and 100 mM.

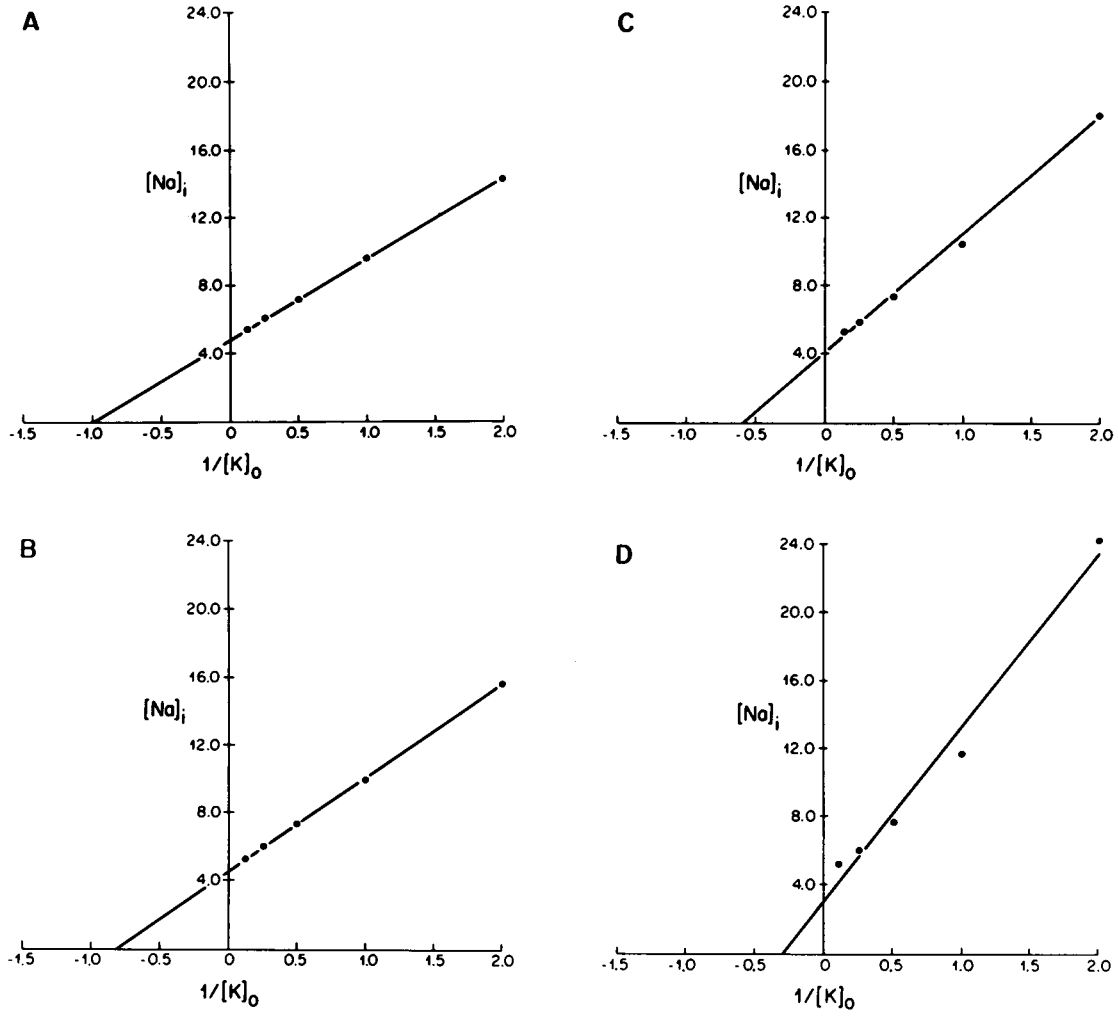

FIGURE 1 Lineweaver-Burk plots constructed from the data in Table I. All data is based on a true K_m for K^+ of 1 mM, and a $[Na^+]_i$ of 6 mM when $[K^+]_o$ = 4 mM. (*A*) The internal Na^+ site is not saturable, (*B*) K_{Na} = 100 mM, (*C*) K_{Na} = 40 mM, and (*D*) K_{Na} = 20 mM. Note that in all cases where the internal Na^+ site is saturable, the K_m obtained from the Lineweaver-Burk plot is an underestimate of the K^+ affinity. Linear regression analysis was used to fit the straight lines.

extrusion mechanisms, the equivalent of Eq. 5 for the saturable internal Na^+ site becomes

$$\frac{[K^+]_o}{K_m + [K^+]_o} \cdot \frac{[Na^+]_i}{[Na^+]_i + K_{Na}} = \text{constant}. \quad (7)$$

Table I (columns B–D) shows the intracellular $[Na^+]$ at different $[K^+]_o$'s, assuming a K_m for $[K^+]_o$ of 1 mM, and a value of $[Na^+]_i$ of 6 mM when the $[K^+]_o$ is 4 mM. The results are calculated for K_{Na} values of 100, 40 and 20 mM $[Na^+]_i$. Fig. 1, *B–D*, shows Lineweaver-Burk plots of this data from which the apparent K_m for $[K^+]_o$ is estimated. In all cases the data are well fit by a straight line. The estimated K_m for K^+ ranges from ~3.4 mM when K_{Na} is 20 mM to ~1.2 mM when K_{Na} is 100 mM. The presence of a saturable internal Na^+ site causes an underestimate of the affinity of the K^+ site. However, as will be demonstrated below, this error in the steady state measurement is smaller than that obtained in similar circumstances by the kinetic measurement.

Kinetic Measurements of the K^+ Affinity of the Na/K Pump Are Badly Distorted by Saturation of the Internal Na^+ Site

Prior to an Na^+ load the preparation is in a steady state, thus sodium entry via the inward background current should equal Na^+ extrusion via the Na^+/K^+ pump (neglecting additional Na^+ extrusion mechanisms)

$$I_{inb} = -B\,I_p. \quad (8)$$

The pump current is related to Na^+ extrusion as in Eq. 2

$$\frac{B\,I_p}{F\,V} = \frac{d\,[Na^+]_i}{dt} = -k\,[Na^+]_i. \quad (9)$$

In response to a sodium load of magnitude $[\Delta\,Na^+]$ there is an increase in the pump rate

$$\frac{B\,(I_p + \Delta\,I_p)}{F\,V} = \frac{d\,[Na^+ + \Delta\,Na^+]_i}{d\,t}$$

$$= -k\,[Na^+ + \Delta\,Na^+]_i. \quad (10)$$

On the assumption that the increment in $[Na^+]_i$ is too small to change the inward background current (Gadsby, 1980), the rate of change of internal $[Na^+]$ is determined by the difference in pump rate between the loaded and control conditions.

$$\frac{d\,[Na^+ + \Delta\,Na^+]_i}{d\,t} - \frac{d\,[Na^+]_i}{d\,t} = \frac{d\,[Na^+]_i}{d\,t} = -k[\Delta\,Na^+]_i. \quad (11)$$

Since this is a first order kinetic scheme, the rate constant is independent of the magnitude of the sodium load. Its value can be obtained by differentiating the pump rate with respect to $[\Delta\,Na^+]_i$

$$\frac{d}{d\,[\Delta\,Na^+]_i}\left(\frac{d\,[\Delta\,Na^+]_i}{d\,t}\right) = \frac{d}{d\,[\Delta\,Na^+]_i}\left(\frac{B\Delta\,I_p}{F\,V}\right) = -k. \quad (12)$$

Now since $k \propto [K^+]_o/(K_m + [K^+]_o)$, the K^+ activation curve can be constructed by measuring the time constants, τ, of the pump current decay at various $[K^+]_o$'s and plotting them against the reciprocal of the $[K^+]_o$. A sample data set of time constants is shown in Table II, column A, assuming a K_m for $[K^+]_o$ of 1 mM and a time constant of pump current decay equal to 75 s in 4 mM $[K^+]_o$. The Lineweaver-Burk plot of this data, which is shown in Fig. 3 *A*, yields the correct K_m of 1 mM.

Now let us consider the analogous situation for a saturable internal Na^+ site. Prior to Na^+ loading:

$$\frac{B}{F\,V} \cdot I_p = \frac{d\,[Na^+]_i}{d\,t} = -\frac{[Na^+]_i \cdot k_{maxx}}{[Na^+]_i + K_{Na}}, \quad (13)$$

and following the Na^+ load

$$\frac{B}{FV}(I_p + \Delta I_p) = \frac{d[Na^+ + \Delta Na^+]_i}{dt}$$

$$= \frac{-\,[Na^+ + \Delta Na^+]_i \cdot k_{maxx} B \Delta I_p}{[Na^+ + \Delta Na^+]_i + K_{Na}\;\; FV}$$

$$= \frac{d[\Delta Na^+]_i}{dt}$$

$$= \frac{-k_{maxx}[Na^+ + \Delta Na^+]_i}{[Na^+ + \Delta Na^+]_i + K_{Na}} + \frac{k_{maxx}[Na^+]_i}{[Na^+]_i + K_{Na}},$$

and

$$= \frac{-k_{max} K_{Na}\,[\Delta\,Na^+]_i}{\left\{\begin{array}{l} K^2_{Na} + [Na^+]^2_i + [Na^+]_i[\Delta\,Na^+]_i \\ \quad + 2K_{Na}\,[Na^+]_i + K_{Na}\,[\Delta\,Na^+]_i \end{array}\right\}}. \quad (14)$$

The apparent rate constant, k', (apparent because the reaction is no longer truly first order) then can be obtained

TABLE II

DEPENDENCE OF THE TIME CONSTANT OF DECAY, τ, ON K_{Na} GIVEN A TRUE VALUE OF 1 mM FOR THE K_m, AND τ = 75 s at $[K^+]_o$ = 4 mM*

$[K^+]_o$	A No saturation τ	B K_{Na} = 100 τ	C K_{Na} = 40 τ	D K_{Na} = 20 τ
mM		*s*		
0.5	180	215	287	518
1.0	120	135	144	179
2.0	90	93	95	102
4.0	75	75	75	75
8.0	67	67	65	64

*Values are calculated for no saturation of the internal Na^+ site and for half-saturation values, K_{Na} = 20 mM, 40 mM, and 100 mM.

by first taking the derivative with respect to $[\Delta\,Na^+]_i$

$$\frac{d}{d[\Delta Na^+]_i}\left(\frac{\Delta I_p B}{FV}\right) = \frac{d}{d[\Delta Na^+]_i}\left[\frac{-k_{maxx}[Na^+ + \Delta Na^+]_i}{[Na^+ + \Delta Na^+]_i + K_{Na}}\right]$$

$$+ \frac{d}{d[\Delta Na^+]_i}\left[\frac{k_{maxx}[Na^+]_i}{[Na^+]_i + K_{Na}}\right]$$

$$= \frac{-k_{maxx} K_{Na}}{([Na^+ + \Delta Na^+]_i + K_{Na})^2}. \quad (15)$$

Then, at any given resting internal $[Na^+]$ the apparent rate constant k' can be determined by letting the load, $[\Delta\,Na^+]_i$, approach zero.

$$\lim_{[\Delta Na^+]_i \to 0}\left[\frac{d}{d[\Delta Na^+]_i}\left(\frac{\Delta I_p B}{FV}\right)\right] = \frac{-k_{maxx} K_{Na}}{([Na^+]_i + K_{Na})^2} = -k'. \quad (16)$$

A plot of $K^2_{Na}/([Na^+]_i + K_{Na})^2$ against $[Na^+]_i/K_{Na}$ is given in Fig. 2. This demonstrates the steep dependence of the apparent rate constant on baseline $[Na^+]_i$. Even when K_{Na} is eight times the value of $[Na^+]_i$ there is a substantial deviation of the apparent rate constant from the true

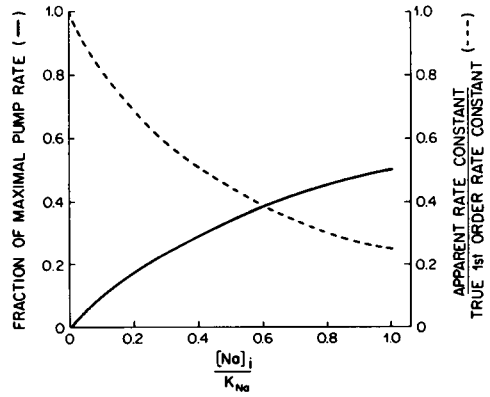

FIGURE 2 A plot of the Na^+ dependence of the Na^+/K^+ pump rate assuming Michaelis-Menten kinetics with half-activation values equal to K_{Na} (solid line). Also shown is the ratio of the apparent rate constant to the true first-order rate constant (dashed line). For more details see text.

first-order rate constant (obtained when $[Na^+]_i = 0$). The values of steady state intracellular sodium for K_{Na}'s of 100, 40, and 20 mM that are given in Table I can be used to calculate the apparent time constants for the kinetic measurements. These time constants are shown in Table II, columns B–D. A pump current decay time constant of 75 s in 4 mM $[K^+]_o$ has been assumed for each K_{Na}. These apparent time constants are plotted in Fig. 3, *B–D*, against the reciprocal of the $[K^+]_o$ to determine the apparent K_m. The values obtained for the K_m are 1.5 mM and 3.0 mM for K_{Na}'s of 100 mM (*B*) and 40 mM (*C*), respectively. At K_{Na} = 20 mM the results clearly deviate from a straight line. The best least squares fit is not interpretable.

The results indicate that kinetic estimates of the K_m for potassium in the presence of a saturable Na^+ site will be severely distorted. These estimates are based on the slope of the relationship between the pump rate and $[Na^+]_i$. However, it is possible that this slope will change dramatically during the course of the decay following a sizable Na^+ load and so not yield the almost perfect exponential decay that is normally observed (Gadsby, 1980; Eisner and Lederer, 1980; Eisner et al., 1981). In the next section we show that if K_{Na} is sufficiently high (≥20 mM), the decay in response to a large Na^+ load (10 mM) is well fit by a single exponential, although the best fit to the apparent rate constant is sensitive to the exact starting level of $[Na^+]_i$.

The Decay of Internal $[Na^+]$ and Pump Current in Response to an Na^+ Load, $[\Delta Na^+]$, Appears Almost Exponential for K_{Na} Sufficiently High

The previous section demonstrated that the rate of decay of the increment in internal $[Na^+]$, $[\Delta Na^+]_i$, depends on the resting level of $[Na^+]_i$. Since the rate of removal of $[\Delta Na^+]_i$ does not obey first-order kinetics, $[\Delta Na^+(t)]_i$

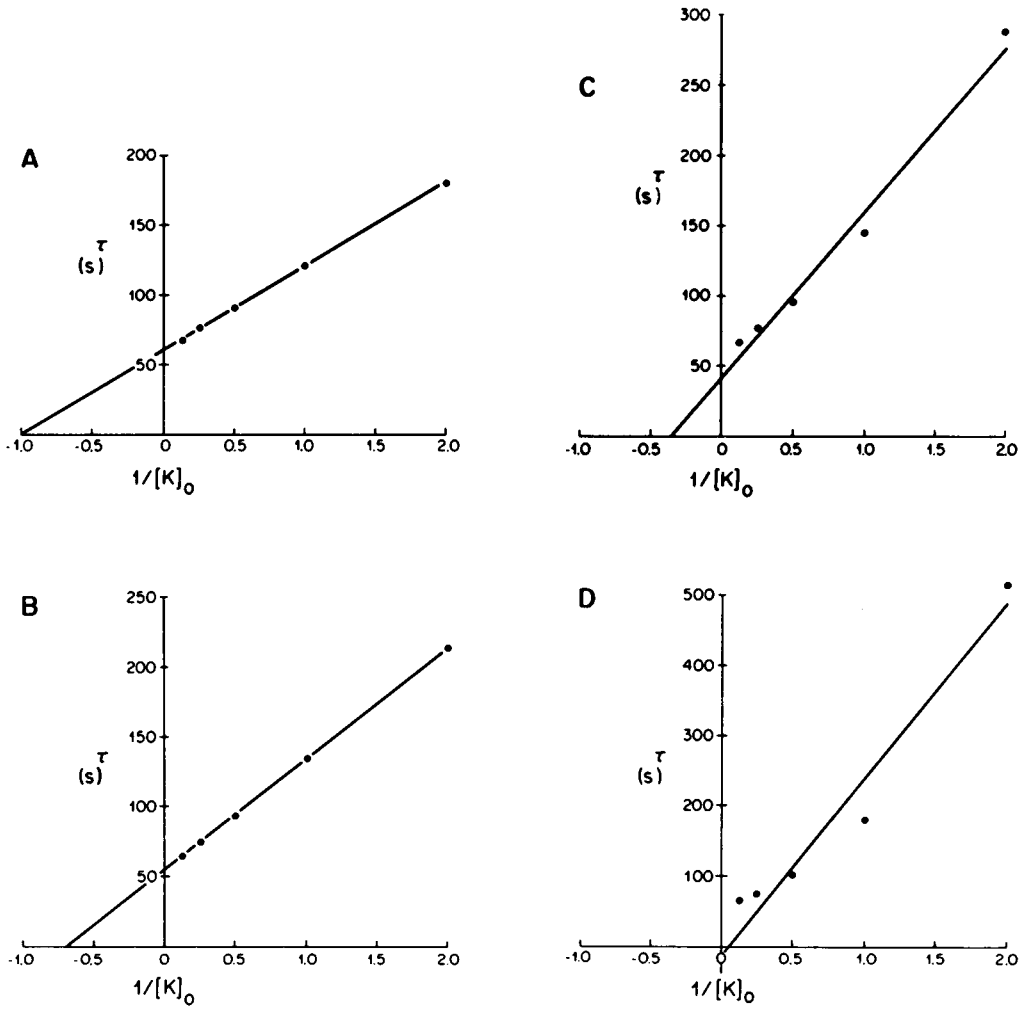

FIGURE 3 Lineweaver-Burk plots constructed from the data in Table II. The K_m for K^+ is assumed to be 1 mM. The rate constant for $[K^+]_o$ = 4 mM is 1/75 s^{-1}. (*A*) No saturation of the internal Na^+ site, (*B*) K_{Na} = 100 mM, (*C*) K_{Na} = 40 mM, and (*D*) K_{Na} = 20 mM. Details of the calculation are provided in the text. Linear regression analysis was used to fit the straight lines.

would be expected to deviate from an exponential, especially for large $[\Delta\ Na^+]_i$ or low K_{Na}.

To examine the decay of the sodium load to baseline levels, it is most convenient to solve Eq. 14 for $[\Delta\ Na^+(t)]_i$. The differential equation is separable and the solution is given in Eq. 17

$$[\Delta Na^+(t)]_i = [\Delta NA(t=0)]_i \exp - \left(\frac{\left(\frac{[Na^+]_i}{K_{Na}} + 1\right)\{[\Delta Na^+(t)]_i - [\Delta Na^+(t=0)]\}_i + k_{maxx}t}{\left\{\frac{[Na^+]_i^2}{K_{Na}} + 2[Na^+]_i + K_{Na}\right\}} \right). \quad (17)$$

As is expected, in the lim $[Na^+]_i \to 0$, lim $[\Delta\ Na^+]_i \to 0$, the equation reduces to

$$[\Delta\ Na^+(t)]_i = [\Delta\ Na^+\ (t=0)]_i \exp\left(\frac{-k_{maxx}t}{K_{Na}}\right) = [\Delta\ Na^+\ (t=0)]_i \exp(-k^*t), \quad (18)$$

where $k_{maxx}/K_{Na} = k^*$ is the apparent first-order kinetic constant. This is the result expected for a first-order kinetic process. Also note that in the lim $[\Delta\ Na^+]_i \to 0$

$$[\Delta\ Na^+(t)]_i = [\Delta\ Na^+(t=0)]_i \exp\left[\frac{-K_{Na}k_{maxx}t}{([Na^+]_i + K_{Na})^2}\right], \quad (19)$$

where $K_{Na}k_{maxx}/([Na^+]_i + K_{Na})^2$ was the expression derived for the apparent rate constant, k', as $[\Delta\ Na^+]_i \to 0$ (Eq. 16). Note further the steep dependence of the apparent rate constant on resting $[Na^+]_i$. When $[Na^+]_i$ is 0, $k_{maxx}K_{Na}/([Na^+]_i + K_{Na})^2 = k_{maxx}/K_{Na} = k^*$. However, when $[Na^+]_i = K_{Na}$ this expression reduces to $k^*/4$. Thus, the apparent rate constant will change dramatically between low resting Na^+ values and values approaching the K_{Na}. This would be expected to result in deviations from the exponential prediction.

Fig. 4 *A–D* shows the decay of a 10 mM Na^+ load from a peak value of 16 mM to the resting level of 6 mM for K_{Na} = 20, 40, 100 mM and for no saturation. The true first-order rate constant is assumed to be 1/75 s^{-1}. For K_{Na} = 20 mM (*D*), 40 mM (*C*) or 100 mM (*B*) a good approximation to a single exponential decay is obtained. However, the best fit to the data does not give 1/75 s^{-1} but 1/150 s^{-1} for K_{Na} = 20 mM, 1/112 s^{-1} for K_{Na} = 40 mM, and 1/89 s^{-1} for K_{Na} = 100 mM. In Fig. 5, decays from two resting $[Na^+]_i$'s are compared for a 10 mM $[\Delta\ Na^+]_i$ with a K_{Na} of 40 mM. When $[Na^+]_i$ is doubled from 6 to 12 mM, the apparent rate constant is reduced from 1/112 to 1/142 s^{-1}. Again, both decays are well fit by a single exponential. Clearly, if $K_{Na} \geq 20$ mM, the decay provides a good fit to the predictions of first order kinetics, but yields the wrong rate constant.

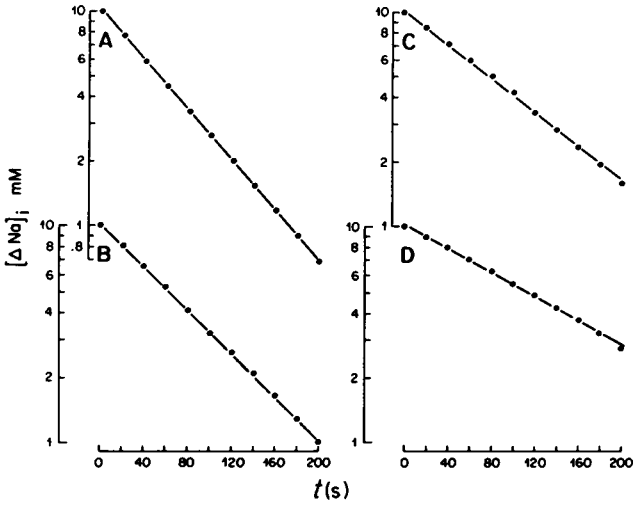

FIGURE 4 The time course of the decay of a 10 mM Na^+ load, $[\Delta Na^+(t)]_i$. The graphs are semilogarithmic plots of $\log[\Delta Na^+(t)]_i$ vs. t according to Eq. 17. The starting levels of $[Na^+]_i$ have been obtained from Table II. (*A*) No saturation for the internal Na^+ site, (*B*) K_{Na} = 100 mM, (*C*) K_{Na} = 40 mM, and (*D*) K_{Na} = 20 mM.

DISCUSSION

The present study was initiated to investigate possible reasons for the substantial difference between kinetic and steady state measures of Na^+/K^+ activity in Purkinje fibers. Eisner et al. (1981) found that the K_m for $[Rb^+]_o$ activation was ~2 mM based on the decay of intracellular Na^+ in response to an Na^+ load, but only 0.8 mM based on steady state estimates in the same sheep Purkinje strand. Average values for kinetic and steady state measures of the K_m were 4 and < 1 mM, respectively. Much of this

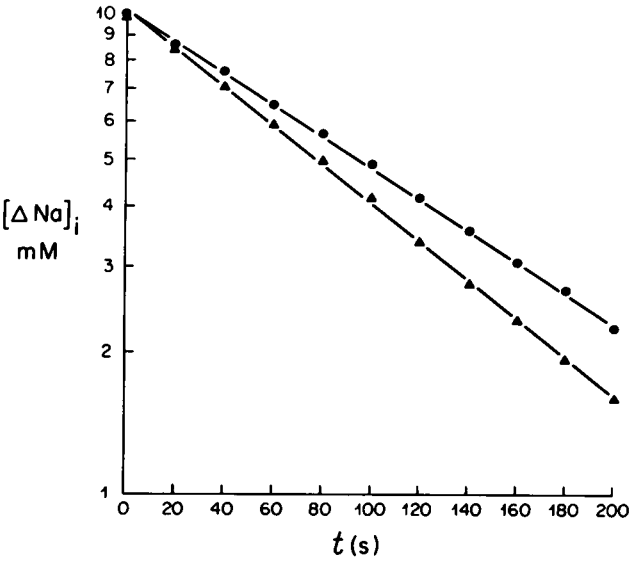

FIGURE 5 The effect of changing baseline $[Na^+]_i$ on the time course of decay of $[\Delta Na^+]_i$ in response to a 10 mM $[Na^+]$ load. ▲, $[Na^+]_i$ = 6 mM; ●, $[Na^+]_i$ = 12 mM.

apparent variability can be explained by proposing a saturable intracellular Na^+ site. If the true K_m were 1 mM, and the K_{Na} were 40 mM, values of 3 mM and 1.7 mM would be obtained for the kinetic and steady state estimates of K_m [Figs. 1 *C* and 3 *C*). The smaller effect of Na^+ saturation on the steady state compared with kinetic measure provides a reason for the relatively high affinity estimates obtained with the steady state measure. Intracellular [Na^+] loads of up to 10 mM were applied by zero [K^+]$_o$ exposures (Eisner et al., 1981). The decay of these sodium loads was always exponential. It is clear from Fig. 4 that an exponential decay, even in response to a 10 mM sodium load, is not sufficient to demonstrate the absence of sodium saturation.

Lower K^+ activation K_m values from steady state analysis have also been reported by Glitsch et al. (1981). Further, the K_m obtained from kinetic analysis by Deitmer and Ellis (1978) by measuring the rate constant of decay rather than the maximum rate of decay of a_{iNa} was 2.6 mM [K^+]$_o$, but analysis of their steady state a_{iNa} values gives a K_m of < 1 mM (Eisner et al., 1981; Gadsby, 1980). Contamination of the kinetic measurements to a greater extent than steady state determinations by the effects of extracellular cleft K^+ (Rb^+) depletion is an alternative possible explanation for the difference between the steady state and kinetic measures (Gadsby, 1980; Eisner et al., 1981; see below).

The effects of saturation of an internal Na^+ site can be observed by altering the baseline [Na^+] concentration and observing the time course of decay in response to a sodium load (Fig. 5). If the increase in baseline sodium is large enough, a decrease in the apparent rate constant should be observed. Only in molluscan neurons has the baseline [Na^+]$_i$ been altered and the response to a sodium load observed (Thomas, 1972). In these experiments baseline Na^+ activity was altered by exposure to Na^+-free Tyrode solution. However, the baseline Na^+ activity only fell from 5 to ~3 mM. No difference in decay rate in response to a sodium load was detected. If the K_{Na} was 40 mM this maneuver would change the decay rate by <9%, a difference that would be difficult to detect experimentally. Similar experiments in canine Purkinje strands in which the preparation was exposed to a Tyrode solution containing 50% of the normal [Na^+] did result in a small (8%) speeding of decay of the pump current (Falk and Cohen, 1983). Although this small difference was in the appropriate direction for Na^+ saturation effects, it was not statistically significant.

The computations in Figs. 1–3 suggest that even for baseline [Na^+]$_i$ well below the K_{Na} for sodium, the apparent rate constant deviates significantly from the true first-order rate constant (observed when [Na^+]$_i$ = 0, and [Δ Na^+]$_i \rightarrow 0$). This dependence of apparent rate constant on the baseline [Na^+]$_i$ implies dose-response curves for glycoside blockage of the sodium pump constructed by examining the pump rate constant for Na^+ extrusion will be in error. The rate of Na^+ extrusion will be slowed due to direct and indirect effects of the drug. The direct effect is to decrease the effective number of pumping sites. The indirect effect is to raise baseline [Na^+]$_i$ and thus slow the rate of sodium extrusion in response to an Na^+ load (Fig. 5).

The analysis presented above might adequately account for the difference in kinetic and steady state estimates of the K_m of the Na^+/K^+ pump in sheep Purkinje fibers. However, it is less certain that the difference between kinetic estimates of the K_m in the sheep Purkinje strand of 3–6 mM, and those in the canine Purkinje strand of 0.9–1.2 mM, can be resolved in this manner (Eisner and Lederer, 1980; Eisner et al., 1981; Glitsch et al., 1981; Gadsby 1980; Falk and Cohen, 1983). One explanation already advanced to explain this difference in kinetic measurements is that the pump current might cause depletion of monovalent cation pump activator in the narrow restricted clefts of the Purkinje fiber (Eisner and Lederer, 1980). This depletion would be larger for the sheep Purkinje strand because it has more restricted extracellular spaces (Hellman and Studt, 1974; Mobley and Page, 1972; Eisenberg and Cohen, 1983). It is difficult to account for the potential independence of the pump current time course by the depletion hypothesis, since changes in holding potential should influence the cleft concentration of the monovalent cation pump activator. One alternative explanation is that the K_{Na} in the Purkinje strand is higher than that in the sheep Purkinje strand, or the baseline [Na^+]$_i$ is lower in the canine than the sheep Purkinje strand. To assess the viability of this alternative hypothesis it would be valuable to have the steady state estimate of the K_m in the canine Purkinje strand, or a comparison of the kinetic estimates of the K_m in isolated canine and sheep Purkinje cells. Unfortunately, neither type of data has yet been reported.

In performing the analysis presented above we made two major assumptions: First, the Na^+/K^+ pump is the only available efflux route for Na^+. Second, the background Na^+ influx is independent of alternative efflux mechanisms. Neither of these two assumptions is likely to be entirely correct, although each has been made in each previous analysis of the Na^+/K^+ pump in Purkinje fibers (Eisner et al., 1981; Gadsby, 1980). Complete blockade of the Na^+/K^+ pump by both cardiotonic steroids and removal of external K^+ results in a rise of intracellular Na^+ activity to only 25 mM (Eisner et al., 1981). Presumably, at these high internal sodium activities alternative extrusion mechanisms (possibly Na^+/Ca^{2+} exchange) become more important than the Na^+/K^+ pump in the maintenance of the intracellular sodium activity at this higher level. However, at lower levels of intracellular [Na^+] it is likely that the majority of the removal of Na^+ in response to a sodium load does occur via the Na^+/K^+ pump,

because there is a striking correlation between the time course of the change in intracellular sodium activity as measured by a sodium-sensitive microelectrode, and the time course of the cardiotonic steroid-sensitive, K^+-activatable pump current (Eisner et al., 1981).

The second assumption requires that the background Na^+ influx be independent of external $[K^+]$. If under physiological conditions the first assumption is obeyed, then the second assumption can be tested by measuring the steady state pump current as a function of the external $[K^+]$. If the background Na^+ influx is K^+-independent, then in the steady state, the pump current should also not depend on external $[K^+]$; intracellular sodium activity should reach a new value that assures a constant pumping rate. Daut (1983), investigating this question in guinea pig ventricular trabeculae, found that steady state pumping current was independent of bathing $[K^+]$ (range 2–4.5 mM).

In this analysis we have considered only the effects of saturation of the internal Na^+ binding site, assuming first-order saturable kinetics for both Na^+ and K^+ activation of the pump. Effects of multiple Na^+ and K^+ binding sites (Garay and Garrahan, 1973; Mullins and Frumento, 1962; Keynes, 1965; Garrahan and Glynn, 1967; Sachs and Welt, 1967), which have also not been observed in cardiac Purkinje strands, have not been considered here. Instead, this treatment focused on the particular assumption that, given a pseudo-linear relationship between pump activity and the change in intracellular sodium activity, Na^+ saturation need not be considered. The analysis leaves all other assumptions intact. Eisner et al. (1981) do point out that multiple binding sites for K^+ activation, and/or failure of the assumptions of sole Na^+ efflux via the Na^+/K^+ pump, and the K^+ independence of the background Na^+ influx, could not account for the differences between their steady state and kinetic measures. We have also performed calculations assuming three equal independent saturable sites for intracellular Na^+ binding. Like Eisner et al. (1981), we conclude that over a range of $[Na^+]_i$ for which near exponential decays of $[Na^+]_i$ in response to a $[Na^+]$ load should occur, multiple Na^+ sites could not eliminate the difference between steady state and kinetic measures. However, one difference from the above analysis does result. In the cubic case, over this range of intracellular $[Na^+]$'s the kinetic measure gives an accurate estimate of the K^+ affinity, while the steady state determination gives a spurious overestimate of the true affinity.

We have also not included in our analysis the actual kinetic mechanism of the Na^+/K^+ pump. Sachs (1977) has pointed out that there will be a relationship between the apparent half-saturation values for external K^+ and internal Na^+ for certain postulated kinetic mechanisms. However, in order to demonstrate such a dependence Sachs (1977) had to rely on a preparation in which both the extracellular $[Na^+]$ and intracellular $[K^+]$ were kept extremely low. In more normal saline solutions, Garay and Garrahan (1973) reported that in human red blood cells the apparent K_m for external K^+ did not vary with the intracellular $[Na^+]$, and Hoffman and Tosteson (1971), using sheep red blood cells, demonstrated that the apparent K_{Na} for intracellular Na^+ did not vary with the extracellular $[K^+]$.

In conclusion, for our analysis of a saturable internal Na^+ site, when $K_{Na} > 20$ mM, both the kinetic and steady state measures of the K_m for $[K^+]_o$ will be in error (the kinetic measure will be more seriously in error). Both kinetic and steady state measures will provide an underestimate of the affinity of the Na^+/K^+ pump for external K^+. Further, an apparent exponential decay in response to an applied sodium load is not sufficient to demonstrate a lack of influence of sodium saturation on estimates of K^+ affinity.

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