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Activation of electrogenic Na^+/K^+ exchange by extracellular K^+ in canine cardiac Purkinje fibers

(sodium pump current/voltage clamp/Michaelis-Menten kinetics/coupling ratio)

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ABSTRACT **Transient increments in sodium pump current were elicited in small voltage-clamped Purkinje fibers suspended in a fast flow system by briefly exposing them to K^+-free fluid, to temporarily inhibit the pump, and then suddenly returning them to K^+-containing fluid. The exponential time course of decay of the current increment provides a measure of the pump rate constant for Na^+ extrusion. The dependence of that rate constant, and of the peak amplitude of the increment in pump current, on the extracellular K^+ concentration was determined. The results indicate: that in cardiac Purkinje cells, as in many other cells, the pump is half-maximally activated by about 1 mM K^+; that the coupling ratio for Na^+/K^+ exchange is independent of either intracellular Na^+ concentration or external K^+ concentration; and that a simple model in which intracellular Na^+ concentration is determined by a passive "leak," and an active extrusion of Na^+, seems sufficient to account for moderate changes in cellular Na^+ concentration.**

The Na^+/K^+ exchange pump in canine cardiac Purkinje fibers is electrogenic (1, 2) and, under appropriate conditions, changes in sodium pump current can be measured directly in small voltage-clamped fibers as changes in holding current (3–5). Transient increases in pump current can be elicited by temporarily inhibiting the pump in K^+-free fluid, to raise the intracellular Na^+ concentration ($[Na]_i$), and then reactivating it by suddenly switching back to K^+-containing fluid. The resulting increment in pump current is seen as a transient positive (outward) overshoot of the steady level of holding current. If the duration of the exposure to K^+-free fluid is lengthened, the peak amplitude of the subsequent increment in pump current is increased, presumably in proportion to the increment in $[Na]_i$ (4). On the other hand, acetylstrophanthidin, a cardiac steroid and specific inhibitor of the sodium pump, abolishes the increment in pump current (4, 6).

We now report that the rate constant for exponential decay of pump current shows a simple Michaelis–Menten type of dependence on the extracellular K^+ concentration ($[K]_o$), half-maximal activation occurring near 1 mM. In addition, the coupling ratio of Na^+/K^+ exchange appears to be independent of changes in either $[Na]_i$ or $[K]_o$. These results have been presented, in preliminary form, to the Biophysical Society (3, 7).

MATERIALS AND METHODS

Small Purkinje fiber bundles, ≤2 mm in length and ≤200 μm in diameter, were dissected from the right ventricles of dog hearts, excised while the animals were under sodium pentobarbital anesthesia. Fibers were suspended in a modified Hodgkin–Horowicz (8) fast flow chamber and impaled with two microelectrodes, a current-passing electrode near the midpoint and a voltage-recording electrode about one third of the distance to the fiber end. Two operational amplifiers were used to clamp the membrane potential at the chosen level (−20 to −45 mV in the present experiments) and to monitor the applied current. Currents were filtered (time constant 100 ms) before being displayed.

The 4 mM K^+ low-chloride solution used in these experiments contained (in mM): Na isethionate, 146; K methylsulfate, 4; Hepes, (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid, pH 7.3), 5; $MgCl_2$, 0.5; Ca methanesulfonate, 0.9–2.7; dextrose, 5.5. K^+ concentration was varied by substituting K methylsulfate for Na isethionate or vice versa. All solutions were oxygenated; experiments were carried out at 37 ± 1°C. Descriptions of the perfusion and recording systems have been published (4, 9).

DESCRIPTION OF MODEL SYSTEM

The results are presented and discussed in terms of the following model system (cf. ref. 6). The sodium pump current, I_p (A), is proportional to the difference between the active pumped efflux of Na^+, A_{Na} (mol s^{-1}), and influx of K^+, A_K (mol s^{-1}), so that $I_p = F(A_{Na} + A_K)$, in which F (C mol^{-1}) is the Faraday constant and efflux of cations is defined as positive. Defining the Na^+/K^+ coupling ratio, r, of the pump as $r = -A_{Na}/A_K$, we have

$$I_p = A_{Na}F\left(1 - \frac{1}{r}\right). \quad [1]$$

The pumped Na^+ efflux, A_{Na}, is assumed to show first-order dependence on $[Na]_i$ (10–15), so that

$$A_{Na} = vk[Na]_i, \quad [2]$$

in which v (cm^3) is the intracellular volume of the preparation, and k (s^{-1}) is the $[K]_o$-dependent rate constant for sodium extrusion. If $[Na]_i$ is determined only by the net active Na^+ efflux, A_{Na}, and net passive Na^+ influx, m_{Na} (mol s^{-1}), then

$$d[Na]_i/dt = -(m_{Na} + A_{Na})/v, \quad [3]$$

and in the steady state, when $d[Na]_i/dt = 0$ and $[Na]_i = [Na]_i(\infty)$,

$$-m_{Na} = A_{Na}(\infty) = vk[Na]_i(\infty), \quad [4]$$

in which $A_{Na}(\infty)$ is the steady-state value of pumped Na^+ efflux. Now, at a fixed potential, m_{Na} may be expected to remain approximately constant in the present experiments (see *Discussion;* cf. refs. 4, 6, 16) so that, combining eqs. 2–4, we have

$$d[Na]_i/dt = -k([Na]_i - [Na]_i(\infty)). \quad [5]$$

In K^+-free fluid, the pump rate constant, k, becomes negligibly small (see *Results*, Fig. 3) so that, from Eq. 2, A_{Na} approaches zero and, from Eq. 3, $d[Na]_i/dt$ rises to a constant value of

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$-m_{Na}/v$. For an exposure to K^+-free fluid of duration T_l (s), therefore, the resulting increment in $[Na]_i$ is

$$\Delta[Na]_i = -m_{Na}T_l/v. \quad [6]$$

When the pump is then reactivated by suddenly returning to the equilibrating $[K]_o$ at time $t = 0$, the increment in $[Na]_i$ declines exponentially, because integration of Eq. 5 yields

$$[Na]_i = [Na]_i(\infty) + \Delta[Na]_i \exp(-kt). \quad [7]$$

If the coupling ratio r does not vary with $[Na]_i$, then from Eqs. 1 and 2 the pump current is always proportional to $[Na]_i$ and is given by

$$I_p = F\left(1 - \frac{1}{r}\right) vk[Na]_i. \quad [8]$$

The steady-state pump current, $I_p(\infty)$, is obtained when $[Na]_i = [Na]_i(\infty)$, thus

$$I_p(\infty) = F\left(1 - \frac{1}{r}\right) vk[Na]_i(\infty). \quad [9]$$

Defining the increment in pump current, $\Delta I_p = I_p - I_p(\infty)$, we have, from Eqs. 7–9,

$$\Delta I_p = F\left(1 - \frac{1}{r}\right) vk\Delta[Na]_i \exp(-kt), \quad [10]$$

showing that the increment in pump current declines exponentially with the rate constant for Na^+ extrusion, k. At time $t = 0$, the initial (maximum or peak) increment in pump current, $\Delta I_p(0)$, is

$$\Delta I_p(0) = F\left(1 - \frac{1}{r}\right) vk\Delta[Na]_i. \quad [11]$$

The total quantity of additional charge, Q (C), moved by the pump during elimination of the increment in $[Na]_i$ is obtained by integration of Eq. 10 between $t = 0$ and $t = \infty$, giving

$$Q = F\left(1 - \frac{1}{r}\right) v\Delta[Na]_i, \quad [12]$$

or

$$Q = \Delta I_p(0)/k. \quad [13]$$

RESULTS

Purkinje fibers were equilibrated to each of several $[K]_o$ levels and, at each level, the pump was switched off for various periods by exposure to K^+-free fluid and then reactivated by suddenly switching back to the equilibrating $[K]_o$. At least 10 min were allowed for equilibration in each K^+-containing fluid, and runs at any given K^+ concentration were usually bracketed by runs at another concentration. The records in Fig. 1 show changes in holding current resulting from 2- to 6-min exposures to zero $[K]_o$, the membrane potential remaining clamped throughout the entire experiment at the more positive of the two levels of resting potential at 4 mM $[K]_o$ (4, 9). The responses in traces a–d were obtained after equilibrating the fiber at 4, 2, 1, and 0.5 mM $[K]_o$, respectively. In each case, on switching to K^+-free fluid, a net increase in inward (negative) current was recorded due to reduction of both pump current and steady-state K^+ current (4). Note, also, that, in the steady state, the inward holding current became larger as $[K]_o$ was reduced from 4 mM to 0.5 mM. At the end of each exposure to K^+-free fluid, on switching back to K^+-containing fluid, a transient increase in pump current was seen. Fig. 1 shows that both the peak amplitude of the increment in pump current and its rate of decay are greatly reduced as the equilibrating level of $[K]_o$ is lowered from 4 mM to 0.5 mM.

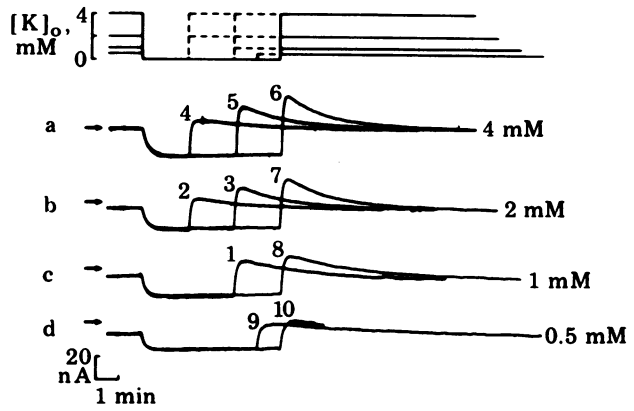

FIG. 1. Superimposed traces of net current recorded from a fiber voltage clamped at −35 mV throughout the $[K]_o$ changes indicated by the upper lines. Traces a, b, c, and d were recorded after equilibrating the fiber to 4, 2, 1, and 0.5 mM $[K]_o$, respectively. Arrows indicate zero current: numbers indicate sequence of solution changes. Fiber D1-24-C, length 2.0 mm, apparent diameter 150 μm; calcium concentration 1.8 mM.

The decaying phases of the pump current increments, ΔI_p, measured from the data in Fig. 1, are shown in the semilogarithmic plots of Fig. 2. Each is well approximated by a single exponential, and at each $[K]_o$ the rate of current decay is independent of the amplitude of the pump current, as shown previously for 4 mM $[K]_o$ (4). However, the rate of decay of the current clearly *is* dependent on $[K]_o$, the average rate constant

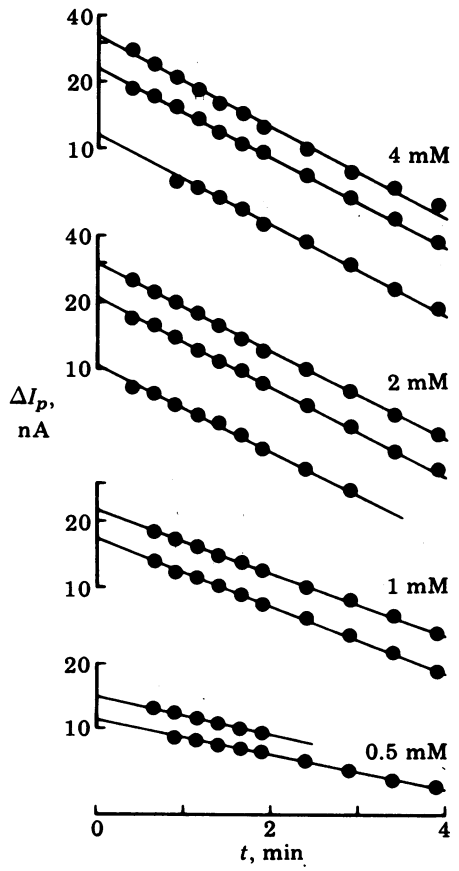

FIG. 2. Semilogarithmic plots of the decay of the transient increments in pump current, ΔI_p, illustrated in Fig. 1. The points were measured from pen recordings; straight lines were fitted to the points by eye. The abscissa shows time, t, from the step change in $[K]_o$ from zero to 4, 2, 1, or 0.5 mM as indicated.

at 0.5 mM $[K]_o$, for example, being only 42% of that at 4 mM $[K]_o$ in the experiment of Fig. 2. This effect of $[K]_o$ was investigated in 16 Purkinje fiber bundles over the $[K]_o$ range 0.5–16 mM. In each experiment, the average rate constant at each $[K]_o$ level, determined from plots like those in Fig. 2, was normalized with respect to the average rate constant obtained at 4 mM $[K]_o$ in that fiber. The mean normalized rate constants, averaged over all 16 experiments, are shown plotted against $[K]_o$ in Fig. 3A. The smooth curve is a hyperbolic function of the form $k = k_{max}\{[K]_o/([K]_o + K_{0.5})\}$, in which k_{max} is the maximal rate constant for decay of pump current, and $K_{0.5}$ is the level of $[K]_o$ for which $k = 0.5\,k_{max}$; in this case, both k and k_{max} have been normalized with respect to the mean rate constant obtained at 4 mM $[K]_o$. The values of normalized k_{max}, 1.20, and of $K_{0.5}$, 0.94 mM, used to construct the curve in Fig. 3A were determined from the least-squares, linear regression fit to the same mean data in the double-reciprocal, Lineweaver–Burk plot shown in Fig. 3B. In experiments on a total of 27 Purkinje fibers (from 13 hearts), the mean time constant for decay of pump current at 4 mM $[K]_o$ was 82 ± 4 s (±SEM), yielding a mean rate constant of 0.012 s^{-1}, or 0.73 min^{-1}. Because, from Fig. 3, k_{max} is found to be 1.2 times the mean k at 4 mM $[K]_o$, the mean maximal rate constant for Na^+ extrusion is calculated to be 0.015 s^{-1}, or 0.88 min^{-1}.

The increment in pump current, ΔI_p, decays exponentially, so the semilogarithmic plots in Fig. 2 can be extrapolated to time $t = 0$ to obtain $\Delta I_p(0)$, the initial increment on pump

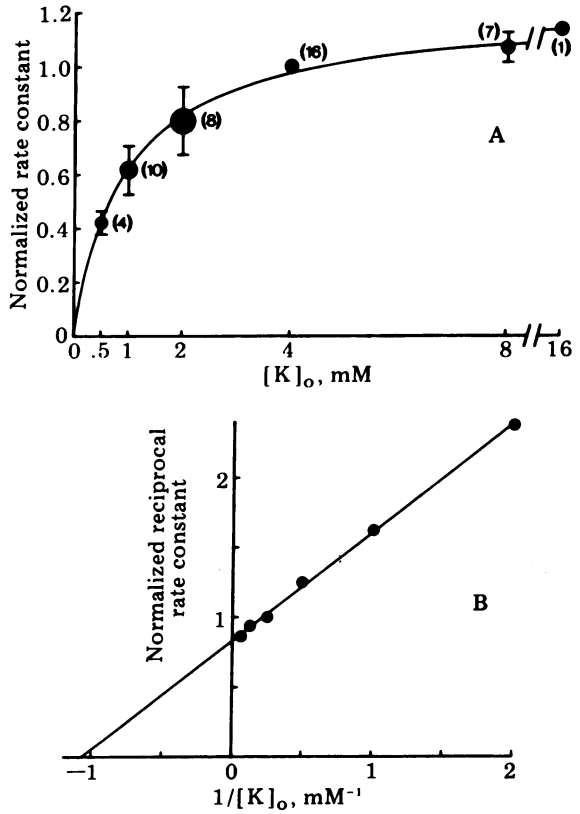

FIG. 3. $[K]_o$ activation of the rate constant for decay of pump current. (A) Mean normalized rate constants are shown by circles with diameters equal to 2 × SEM; the vertical bars indicate ± SD, and the numbers in parentheses give the number of experiments represented by each mean. The smooth curve is a hyperbolic function which saturates at a normalized rate constant of 1.20 and is half-maximal at $[K]_o$ = 0.94 mM (see text). (B) Double-reciprocal plot of same mean data as shown in A. The straight line shows the least-squares regression fit to the points (coefficient of determination 0.999), from which the parameters used to construct the curve in A were determined.

reactivation (4). The values of $\Delta I_p(0)$ so obtained from Fig. 2 are shown in Fig. 4, plotted against the duration, T_l, of the preceding Na^+-loading period in K^+-free fluid. The lines drawn through the points obtained at each $[K]_o$ indicate that the relationship between $\Delta I_p(0)$ and T_l is approximately linear at all levels of $[K]_o$, as previously found for 4 mM $[K]_o$ (4). From Eq. 6, the increment $\Delta[Na]_i$ is expected to increase linearly with T_l so that if the Na^+/K^+ coupling ratio, r, is independent of $[Na]_i$, then Eq. 11 predicts that, at any level of $[K]_o$, the initial increment, $\Delta I_p(0)$, should be proportional to the loading time, T_l. The results of Fig. 4 clearly support this prediction. At a given $[K]_o$, because $\Delta I_p(0)$ *is* proportional to T_l (Fig. 4), and the rate constant k *is* independent of T_l (Fig. 2), then the increment in charge extruded by the pump, $Q = \Delta I_p(0)/k$ (eq. 13), must increase linearly with loading time T_l (see also ref. 4). This observation further suggests that r is, indeed, independent of $[Na]_i$ (see Eq. 12). An additional argument for r being independent of $[Na]_i$, based on the exponential decline of ΔI_p and the presumed, parallel exponential decline of the increment in $[Na]_i$ (cf. refs. 12–15, 17) has been discussed previously (4).

If r is independent of *both* $[Na]_i$ and $[K]_o$, then Eq. 11 predicts that, after a given duration, T_l, of exposure to K^+-free fluid, which causes a given increment in concentration, $\Delta[Na]_i$, the amplitude of $\Delta I_p(0)$ measured at different $[K]_o$ levels should vary in proportion to the pump rate constant k. This is confirmed by comparing the relative slopes of the lines drawn in Fig. 4 with the normalized rate constants obtained, for the same fiber, from Fig. 2: the lines in Fig. 4 have relative slopes, for 4, 2, 1, and 0.5 mM $[K]_o$, of 1.0, 0.94, 0.72, and 0.41, respectively, while the corresponding ratios for the average rate constants in the same experiment (Fig. 2) are 1.0, 0.97, 0.74, and 0.42.

Further evidence that the coupling ratio is independent of $[K]_o$ comes from a consideration of the additional quantity of charge, Q, generated at different levels of $[K]_o$ during extrusion of the same increment in concentration, $\Delta[Na]_i$. If $\Delta[Na]_i$ is independent of the equilibrating level of $[K]_o$, as required by Eq. 6 (see *Discussion*), then from Eq. 12, Q will similarly be independent of $[K]_o$ unless the coupling ratio, r, changes. For each $[K]_o$, Q was determined as $\Delta I_p(0)/k$ (Eq. 13) from the data in Fig. 2 and plotted against T_l in Fig. 5. All the points fall on or near the same straight line irrespective of the equilibrating $[K]_o$. As already mentioned, for a given $[K]_o$, a constant slope,

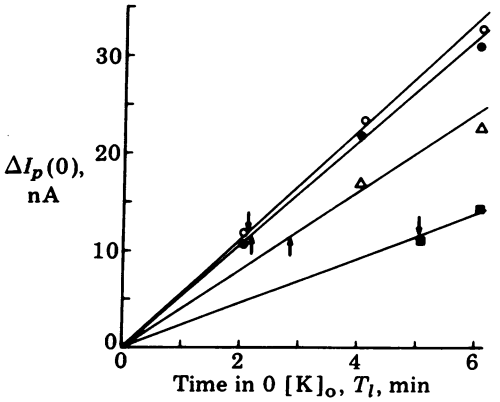

FIG. 4. Plots of initial, maximum, increments in pump current, $\Delta I_p(0)$, at different $[K]_o$ levels, from the experiment of Figs. 1 and 2, versus the duration, T_l, of the preceding exposure to zero $[K]_o$. $\Delta I_p(0)$ was obtained by extrapolation of the semilogarithmic plots in Fig. 2 to time $t = 0$. $[K]_o$ levels are: ○, 4 mM; ●, 2 mM; △, 1 mM; ■, 0.5 mM. Arrows indicate values of $\Delta I_p(0)$ for durations $T_l = 1/k$, in which k is the average rate constant for pump current decay obtained for this fiber at the appropriate $[K]_o$ (see text).

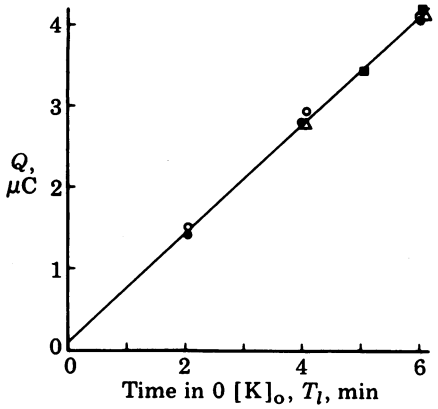

FIG. 5. Plot of additional quantity of charge, Q, transferred by the pump during extrusion of the increments in $[Na]_i$, for the experiment of Figs. 1, 2, and 4, versus the duration, T_l, of the preceding Na^+-loading period. Q was determined as $\Delta I_p(0)/k$ from the data in Fig. 2. $[K]_o$ levels: ○, 4 mM; ●, 2 mM; △, 1 mM; ■, 0.5 mM. The straight line is a least-squares fit (coefficient of determination 0.998).

Q/T_l, implies that the coupling ratio is independent of $[Na]_i$; that the slope Q/T_l remains unchanged as $[K]_o$ is varied shows that coupling ratio is independent *both* of $[K]_o$ *and* of $[Na]_i$. Comparable results were obtained in 12 other preparations: thus, after a given Na^+-loading period (T_l range 1.5–3 min), values for Q [obtained as $\Delta I_p(0)/k$] at equilibrating K^+ concentrations of 8, 2, and 1 mM were normalized with respect to the values found in the same fiber at 4 mM $[K]_o$, and the mean ratios (±SD) were: 8 mM, 0.99 ± 0.17 (n = 4); 4 mM, 1.0 (n = 15); 2 mM, 1.04 ± 0.08 (n = 7); 1 mM, 0.96 ± 0.10 (n = 10).

A close correlate of the above arguments is that Eqs. **6** and **11** predict that the initial pump current transient, $\Delta I_p(0)$, will have the same size at different equilibrating $[K]_o$ levels, immediately following exposures to K^+-free fluid whose durations are related by the inverse ratio of the rate constants corresponding to those steady $[K]_o$ levels. The arrows in Fig. 4 point to interpolated (or extrapolated) values of $\Delta I_p(0)$ for Na^+-loading periods of durations $T_l = 1/k$ (using average values of k determined from Fig. 2) appropriate for each $[K]_o$; as the model predicts, these increments have approximately the same size. In experiments on 10 fibers, the ratios of the current increments, $\Delta I_p(0)$, obtained at different $[K]_o$ levels, similarly interpolated for K^+-free exposures of durations $T_l = 1/k$, when normalized to the values at 4 mM $[K]_o$, averaged (±SD): 8 mM, 1.01 ± 0.02 (n = 2); 4 mM, 1.0 (n = 10); 2 mM, 1.01 ± 0.04 (n = 6); 1 mM, 0.98 ± 0.08 (n = 7).

DISCUSSION

Glitsch *et al.* (18) found that the electrogenic Na^+/K^+ pump in guinea pig atria was half-maximally activated at a $[K]_o$ of 1.5 mM, in close agreement with the present results. In Na^+-loaded sheep Purkinje fibers, however, Deitmer and Ellis (13) found that the rate of decline of intracellular Na^+ activity was half-maximal near 10 mM $[K]_o$. A similarly high $K_{0.5}$ value, of about 6 mM, was recently obtained for Rb^+ activation of the rate constant for pump current decay in voltage-clamped sheep Purkinje fibers (6), suggesting that the $K_{0.5}$ for K^+ activation in that study would also have been near 6 mM, because Rb^+ and K^+ are approximately equipotent in activating the pump (16, 19). A likely explanation for these apparently large $K_{0.5}$ values for pump activation by K^+ or Rb^+ in sheep Purkinje fibers is that, during recovery from Na^+-loading, net uptake of K^+ or Rb^+ from the narrow clefts between adjacent cells lowers the K^+ or Rb^+ concentration there considerably below that in the bulk bathing fluid. Indeed, Eisner and Lederer (6) have demonstrated, albeit indirectly, such depletion of extracellular K^+ under the conditions of their experiments and have suggested, therefore, that quantitatively similar depletion of Rb^+ must also be expected to occur. That such K^+ depletion is much less severe in small canine Purkinje fibers such as those used for the present study is suggested by the low $K_{0.5}$ value obtained (1 mM) for pump activation (see also ref. 2), and by preliminary electrophysiological (20) and morphological (T. D. Pham and D. C. Gadsby, unpublished) studies, both of which indicate relatively wide spaces between adjacent cells. For example, Cohen et al. (20) reported that the slow decrease in inward K^+ current during hyperpolarizing voltage-clamp pulses, which results from extracellular K^+ depletion, represents a much smaller fraction of the final, steady inward current in canine than in sheep Purkinje fibers. Of course, any K^+ depletion that occurred during the present study would not help explain the apparent difference in $[K]_o$ sensitivity of the Na^+/K^+ pump in sheep versus that in dog Purkinje fibers. In fact, because extracellular K^+ depletion cannot be entirely ruled out in experiments with small canine Purkinje fibers, the estimate of 1 mM for $K_{0.5}$ must be considered an upper limit. The physiological significance of such a low $K_{0.5}$ value is that at normal serum K^+ concentrations (4–5 mM) the pump is practically saturated with respect to $[K]_o$, so that the pump rate is regulated predominantly by changes in $[Na]_i$ (21).

In the steady state, when Na^+ and K^+ fluxes are in balance, K^+ depletion should be negligibly small even in sheep Purkinje fibers (unless there is considerable local specialization of the cell surface membrane) and, in support of this prediction, the *steady-state* data of Deitmer and Ellis (13) are consistent with the present results from small canine Purkinje fibers. Thus, ignoring the less than 10% variation in passive Na^+ influx these authors found at different $[K]_o$ levels, $[Na]_i(\infty)$ can be expected (from Eq. **4** above) to vary inversely with the pump rate constant, k. Figure 2 of Deitmer and Ellis (13) shows that $[Na]_i(\infty)$ changed little between 6 and 12 mM $[K]_o$, indicating that k is already maximal at 6 mM, but that $[Na]_i(\infty)$ was almost doubled at 1 mM $[K]_o$, suggesting that the rate constant was then roughly half maximal. That this simplified steady-state analysis differs markedly from that of the Na^+-loaded fibers is presumably due to the considerable extracellular K^+ depletion occurring during enhanced Na^+ extrusion in sheep Purkinje fibers (6).

An important assumption in the model used here is that, at a constant membrane potential, the passive Na^+ flux, m_{Na}, remains approximately constant in spite of significant changes in $[K]_o$ and $[Na]_i$. This assumption can be shown to be valid for a passive Na^+ flux that obeys the constant-field flux equation (22, 23),

$$m_{Na} = P_{Na}\frac{VF}{RT}\frac{[Na]_o - [Na]_i\exp(VF/RT)}{1-\exp(VF/RT)}, \qquad [14]$$

in which V is membrane potential, F the Faraday constant, R the gas constant, T the absolute temperature, and P_{Na} the membrane permeability coefficient for Na^+. Considering changes in $[Na]_i$ at a fixed membrane potential (P_{Na} assumed constant), we find that at 37°C, when $[Na]_o$ = 150 mM and V = −35 mV, a 6-fold rise in $[Na]_i$ from 5 mM to 30 mM reduces m_{Na} by less than 5%. Our experimental results support such constancy of m_{Na}. Thus, because the exponential decline of the increments in both $[Na]_i$ and pump current indicates that the coupling ratio, r, is independent of $[Na]_i$ (12, 14, 15), the linear increases in both increments, $\Delta I_p(0)$ and Q, with increasing duration, T_l, in K^+-free fluid (ref. 4; present study, Figs. 4 and 5), suggest that the increment in Na^+ concentration, $\Delta[Na]_i$, increases linearly with T_l, in accordance with Eq. **6**. Strictly,

for Eq. 6 to hold, the pump rate should be zero in K^+-free fluid. In fact, Na^+ extrusion probably does cease at zero $[K]_o$ in our experiments, because the net current in K^+-free fluid is not significantly changed when a maximal concentration of cardiac steroid is present (see Fig. 2 of ref. 4). If Na^+ extrusion ceases at zero $[K]_o$, then $[Na]_i$ should double during a Na^+-loading period of duration $T_l = 1/k$, because Eqs. 4 and 6 show that, for that particular case, $\Delta[Na]_i = [Na]_i(\infty)$. For example, for a fiber equilibrated at 4 mM $[K]_o$, $[Na]_i$ will be doubled after exposure to K^+-free fluid for, on average, 82 s, the mean reciprocal rate constant for 4 mM $[K]_o$ (see ref. 2, page 834, for a similar conclusion based on different data). Because the plots at 4 mM $[K]_o$ in Figs. 4 and 5 remain approximately linear for loading periods lasting up to 3 reciprocal rate constants, we conclude that m_{Na} remains approximately constant even when $[Na]_i$ is raised to 4 times its "normal" resting level at 4 mM $[K]_o$. This conclusion is supported by the approximate linearity of the corresponding plots for 0.5 mM $[K]_o$ over loading periods roughly equivalent to one reciprocal rate constant at 0.5 mM $[K]_o$; thus, from Fig. 3, the normalized rate constant at 0.5 mM $[K]_o$ is about 0.4 so that, from Eq. 4, the steady-state intracellular Na^+ concentration at 0.5 mM $[K]_o$ should be about 2.5 times that at 4 mM $[K]_o$, and this concentration would be further doubled during the prolonged exposure to zero $[K]_o$.

We can use our measurements of transient increments in pump current to estimate the steady-state level of pump current, $I_p(\infty)$. Because $\Delta[Na]_i = [Na]_i(\infty)$ after a Na^+-loading period of duration $T_l = 1/k$, then, from Eqs. 9 and 11, the resulting initial increment in pump current $\Delta I_p(0)$ should equal the steady-state level $I_p(\infty)$. In Fig. 4, the appropriate values of $\Delta I_p(0)$ corresponding to loading periods of durations equivalent to the reciprocal rate constants obtained for 4, 2, 1, and 0.5 mM $[K]_o$ are marked by arrows. These currents are equivalent to $I_p(\infty)$ after equilibration at each $[K]_o$ level and they are all approximately the same. In other words, at a fixed membrane potential the steady-state pump current is independent of $[K]_o$ and this is what the model predicts because, as long as m_{Na} is constant, then, from Eqs. 4 and 9, $I_p(\infty)$ must also be constant. In that case, the increase in the *steady* inward holding current associated with lowering of $[K]_o$, noted in connection with Fig. 1, can, presumably, be attributed entirely to reduction in outward K^+ current.

The reasonable agreement of experimental results with predictions of a simple model provides an encouraging basis for detailed investigations of the mechanism of action of a variety of agents that are believed to modify Na^+/K^+ pump activity in the heart.

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