Proc. Natl. Acad. Sci. USA
Vol. 85, pp. 3412–3416, May 1988
Biophysics

Potassium translocation by the Na^+/K^+ pump is voltage insensitive

(active transport/Na^+/K^+-ATPase/pump current/transport kinetics)

ANTHONY BAHINSKI, MASAKAZU NAKAO*, AND DAVID C. GADSBY†

Laboratory of Cardiac Physiology, The Rockefeller University, New York, NY 10021-6399

Communicated by Frank Brink, Jr., February 5, 1988 (received for review November 27, 1987)

ABSTRACT **The voltage dependence of steady and transient changes in Na^+/K^+ pump current, in response to step changes in membrane potential, was investigated in guinea pig ventricular myocytes voltage clamped and internally dialyzed under experimental conditions designed to support four separate modes of Na^+/K^+ pump activity. Voltage jumps elicited transient pump currents when the pump cycle was running forward or backward, or when pumps were limited to Na^+ translocation, but not when they were made to carry out K^+/K^+ exchange. This result indicates that K^+ translocation involves no net charge movement across the membrane field and is therefore voltage insensitive. The transient pump currents seen during Na^+/K^+ transport demonstrate that both forward and reverse pump cycles are rate limited not by the voltage-dependent step but by a voltage-independent step, probably K^+ translocation. These findings severely constrain kinetic models of Na^+/K^+ pump activity.**

An electrogenic ion transporter, by definition, moves net charge across the cell membrane and is therefore expected to have a voltage-dependent transport rate, at least within a limited voltage range. The ubiquitous Na^+/K^+ pump is probably the best-studied electrogenic transporter, and cell membrane potential has recently been shown to modulate Na^+/K^+ pump rate whether the pump cycle runs forward or backward (1), but which reaction steps confer voltage sensitivity on the cycle, and how, is not yet fully resolved. Thus, although recent measurements of transient charge movements during partial reactions of the Na^+/K^+ pump in the absence of external K^+ (2–4) indicate that Na^+ translocation includes a voltage-dependent step (cf. refs. 5, 6), the contribution of this step to the voltage dependence of the Na^+/K^+ pump reaction cycle remains unclear because Na^+ translocation is not thought to limit the rate of the cycle (refs. 7, 8; but cf. ref. 6). Moreover, the possibility that K^+ translocation includes a voltage-sensitive step (9–11) remains controversial (1, 6, 12). To address these issues, we measured both transient (3) and steady-state (13) Na^+/K^+ pump currents in guinea pig ventricular myocytes, voltage clamped in the whole-cell recording mode (14) using wide-tipped patch pipettes and a device for exchanging the solution inside the pipette (15). The results show that K^+ translocation is voltage insensitive and suggest that the Na^+/K^+ pump reaction cycle includes only a single voltage-dependent step which, however, does not limit the rate of either the forward or the reverse transport cycle. These results have been presented in brief elsewhere (16).

MATERIALS AND METHODS

Hearts were rapidly excised from guinea pigs (300–500 g) fully anesthetized with sodium pentobarbital (≈50 mg/kg, i.p.), their aortas were cannulated, and retrograde perfusion of the coronary arteries was begun, first with normal Tyrode's solution (145 mM NaCl/5.4 mM KCl/1.8 mM $CaCl_2$/0.5 mM $MgCl_2$/5.5 mM dextrose/5 mM Hepes·NaOH, pH 7.4), then with nominally Ca^{2+}-free Tyrode's solution for 3 min, and then for ≈15 min with the latter solution containing collagenase (type I, Sigma) at 0.5 mg/ml. The collagenase was washed out of the partially digested heart, which was cut open and kept in a high K^+/low Ca^+ medium (17). Cells harvested from fragments of myocardium were allowed to settle onto the glass coverslip forming the bottom of the experimental chamber on the movable stage of an inverted microscope (Nikon Diaphot), before beginning superfusion with normal Tyrode's solution at 36°C. Giga-ohm seals were obtained with wide-tipped (≈5 μm), fire-polished, pipettes (resistance, ≈1 MΩ) filled with normal Tyrode's solution that was exchanged (15) just before rupture of the cell membrane for pipette solution (50 mM NaOH/≈85 mM CsOH/85 mM aspartic acid/5 mM pyruvic acid/2 mM $MgCl_2$/10 mM MgATP/5 mM $Tris_2$ creatine phosphate/20 mM tetraethylammonium chloride/5.5 mM dextrose/10 mM EGTA/10 mM Hepes, pH 7.4). The voltage-clamped cell was then superfused with modified Ca^{2+}-free Tyrode's solution (150 mM NaCl/2.3 mM $MgCl_2$/2 mM $BaCl_2$/0.5 mM $CdCl_2$/5.5 mM dextrose/5 mM Hepes·NaOH, pH 7.4). Variations of these intracellular and extracellular solutions, as specified in text and legends, were used to sustain four modes of Na^+/K^+ pump activity (e.g., ref. 18), namely Na^+/Na^+ exchange, forward or backward Na^+/K^+ exchange, or K^+/K^+ exchange. The osmolarity of all solutions was ≈300 mosM, and all solutions were designed to minimize ion-channel currents (3, 13) and Na^+/Ca^{2+} exchange current (19). Na^+/K^+ pump current was determined as the difference between whole-cell current recorded in the absence and in the presence of 0.5–2 mM strophanthidin. Strophanthidin was added from a 0.5 M solution in dimethyl sulfoxide; control measurements showed that up to 0.5% (by volume) dimethyl sulfoxide had no effect on membrane currents (see Fig. 3*C*).

Current and voltage signals were low-pass filtered at 2 kHz (6-pole Bessel filter), digitized (12-bit resolution) on line at 8 kHz, and stored in a computer for later analysis. Up to 55% of the series resistance between pipette interior and cell membrane was compensated by summing a fraction of the clamp output to the command potential. The voltage clamp amplifier was connected to the pipette interior and to the experimental chamber via 3 M KCl-filled half cells to minimize voltage errors due to liquid-junction potentials. Accord-

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: $[E_1]$, steady-state concentration of enzyme intermediate 1; $[E_2]$, steady-state concentration of enzyme intermediate 2; $[K^+]_o$, external K^+ concentration; $[Na^+]_o$, external Na^+ concentration; $[K^+]_i$, internal K^+ concentration; $[Na^+]_i$, internal Na^+ concentration.

†To whom reprint requests should be addressed at: Laboratory of Cardiac Physiology, The Rockefeller University, 1230 York Avenue, New York, NY 10021-6399.

*Present address: Department of Anesthesiology, Hiroshima University School of Medicine, Hiroshima 734, Japan.

ingly, no corrections have been applied to the results presented here.

RESULTS

Fig. 1 *Aa* shows superimposed records of strophanthidin-sensitive currents in a cell equilibrated with 50 mM Na^+/10 mM ATP pipette solution and bathed in K^+-free solution containing 150 mM Na^+. Lack of external K^+ interrupted the pump's transport cycle, as confirmed by the absence of steady strophanthidin-sensitive current at any voltage (Fig. 1 *Aa* and *B*), and restricted pump activity to Na^+ translocation. The pump nevertheless mediated voltage-dependent, transient charge displacements (Fig. 1*A*) that decayed exponentially with voltage-dependent rate constants, were oligomycin sensitive, and required internal ATP as well as internal and external Na^+ (3). The transient currents indicate that Na^+ translocation involves net charge movement within the membrane field (2–6) and so has voltage-dependent reaction rates (10, 11, 20).

Stepwise addition of external K^+ activated the Na^+/K^+ transport cycle in a concentration-dependent manner, as evident from the increasing levels of steady outward pump current (Fig. 1 *A* and *B*) whose monotonic voltage dependence is shown in the pump current–voltage (I–V) relationships plotted in Fig. 1*B*. As steady current was increased, the area under the transient currents (i.e., the quantity of charge displaced) was diminished (Fig. 1*A*), but even during the strong, almost maximal activation of the forward Na^+/K^+ pump cycle by 5.4 mM $[K^+]_o$, voltage steps still clearly elicited small transient pump currents that preceded the new steady pump current levels. These transient currents reflect voltage-dependent redistribution of enzyme intermediates involved in the forward Na^+/K^+ pump cycle. Specifically, they indicate that the rate-limiting step in the cycle occurs after the charge-translocating step, which is the step instantaneously perturbed by a voltage jump, so that the initial increment of pump current decays due both to depletion of the intermediate entering that voltage-sensitive step and to accumulation of its product (see *Discussion*; cf. ref. 21).

To drive the Na^+/K^+ pump reaction cycle backwards (16, 22–24) (Fig. 2) we steepened the transmembrane Na^+ and K^+ gradients by removing internal Na^+ and external K^+ and setting $[Na^+]_o$ at 150 mM and internal K^+ concentration $[K^+]_i$ at 145 mM, and we included 5 mM ATP, 5 mM ADP, and 5 mM inorganic phosphate (P_i) in the pipette solution. Strophanthidin then caused an *outward* shift of holding current (Figs. 2*A* and 3*A*) reflecting abolition of steady *inward pump current*. Steady-state, whole-cell I–V relationships determined before, during, and after the exposure to strophanthidin are shown in Fig. 2*C*. The resulting strophanthidin-sensitive I–V relationship (Fig. 2*D*) reveals that, like the outward pump current in Fig. 1*B*, inward current generated by the backward-running Na^+/K^+ pump also had a monotonic voltage dependence: it increased in amplitude as membrane potential was made more negative, from an extremely small size at +40 mV toward an apparent plateau level near −100 mV, and no region of negative slope conductance (23) was evident.

Small transient pump currents were found to precede establishment of the new steady current levels (Figs. 2*B* and 3*B*) following step changes in membrane potential, demonstrating that also in the reverse Na^+/K^+ transport cycle the rate-limiting step occurs *after* the voltage-sensitive step.

The steady *inward* pump current shown in Figs. 2 and 3 confirmed that the Na^+/K^+ pump reaction was continuously cycling backward and so gave us confidence that 5 mM P_i and

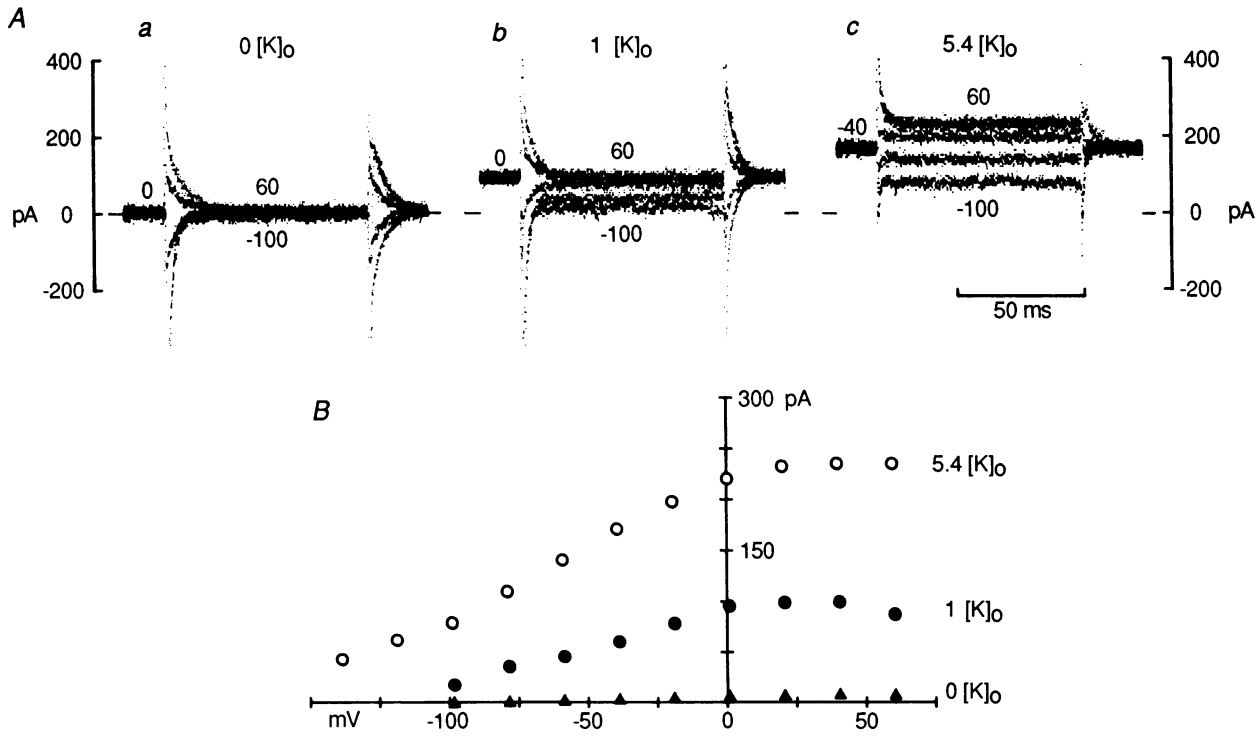


FIG. 1. External K^+ concentration ($[K^+]_o$) dependence of transient and steady pump currents from a cardiac cell dialyzed with 50 mM Na^+/10 mM ATP pipette solution and exposed to 145–150 mM external Na^+ concentration ($[Na^+]_o$). (*A*) Superimposed records of strophanthidin-sensitive currents during 80-ms voltage pulses to +60, +20, −20, −60, and −100 mV, obtained by computer subtraction of whole-cell current traces recorded in the presence of 0.5 mM strophanthidin from the corresponding current traces recorded in its absence ≈1 min earlier. Holding potential: *a* and *b*, 0 mV; *c*, −40 mV. $[K^+]_o$, varied by substituting K^+ for Na^+, was 0 (*a*), 1 (*b*), or 5.4 mM (*c*). (*B*) Na^+/K^+ pump current–voltage relationships from the results in *A*. Ordinate, steady levels of strophanthidin-sensitive currents obtained by averaging digitized values over the same 24-ms period near the end of each pulse. Abscissa, membrane potential during the pulse. ▲, 0 mM $[K^+]_o$; ●, 1 mM $[K^+]_o$; ○, 5.4 mM $[K^+]_o$. Total cell capacitance, 218 pF. Initial pipette resistance, 1.0 MΩ; 0.8 MΩ of the 1.7 MΩ series resistance between pipette interior and cell membrane was compensated.

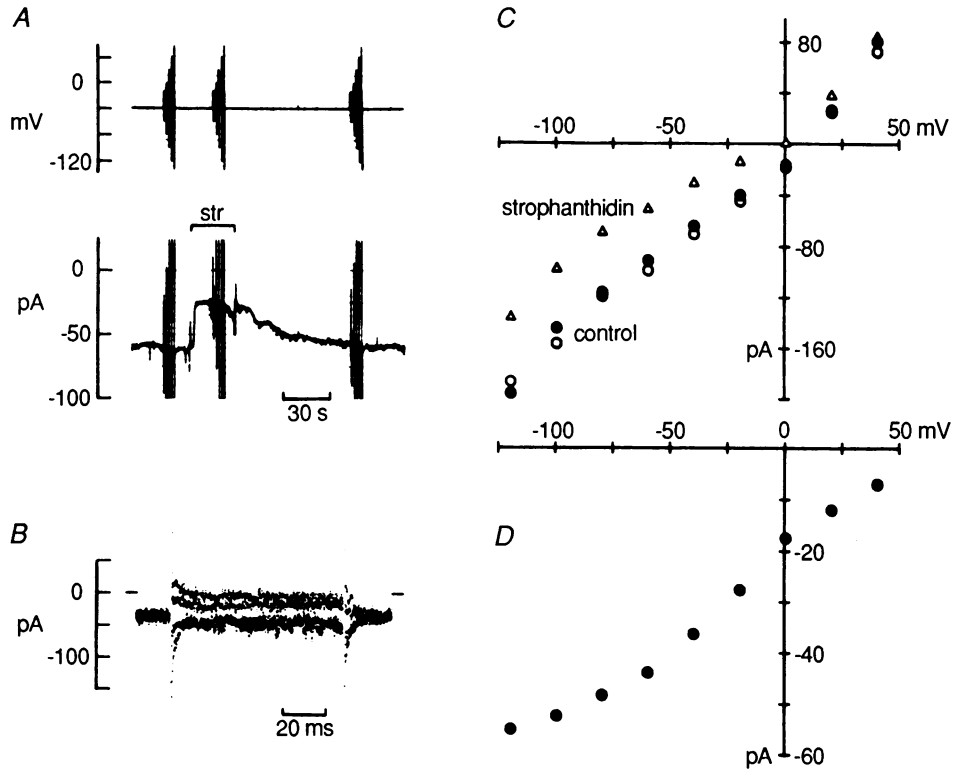


FIG. 2. Voltage dependence of inward current generated by the backward-running Na^+/K^+ pump. (*A*) Chart recording of membrane potential (*Upper*) and membrane current (*Lower*); holding potential, −40 mV. The horizontal bar marks exposure to 0.5 mM strophanthidin (str). The K^+-free external solution was as described but contained 5 mM Ba^{2+}; the internal solution was as described but was free of Na^+, Cs^+, and creatine phosphate, and contained instead 145 mM K^+, 5 mM MgATP, 5 mM $Tris_2$ ADP, and 5 mM phosphate. (*B*) Superimposed records of strophanthidin-sensitive currents for 80-ms pulses to +40, 0, −60, and −100 mV, obtained by subtracting each trace recorded in the presence of strophanthidin from the average of control traces recorded during pulses to the same potential just before and just after the exposure to strophanthidin. (*C*) Whole-cell current–voltage relationships from the experiment in *A*, determined before (○), during (△), and after (●) exposure to strophanthidin. Ordinate, steady current levels measured by averaging points over a fixed 8-ms period near the end of each pulse; abscissa, membrane potential. (*D*) Current–voltage relationship of the backward-running Na^+/K^+ pump (from ref. 16). Ordinate, steady levels (measured as in *C*) of the strophanthidin-sensitive currents represented in *B*; abscissa, membrane potential. Total cell capacitance, 161 pF. Initial pipette resistance, 1.3 MΩ; 0.8 MΩ of the 1.9 MΩ series resistance was compensated.

145 mM $[K]_i$ were adequate to sustain reverse K^+ translocation via the Na^+/K^+ pump. We already knew that 5.4 mM $[K^+]_o$ and millimolar levels of ATP were adequate to drive forward K^+ translocation (Fig. 1). So, to force the pump to carry out K^+/K^+ exchange, we interrupted backward-running pump experiments, as illustrated in Fig. 3, by replacing all external Na^+ with *N*-methyl-D-glucamine$^+$ and simultaneously adding 5.4 mM external K^+, while retaining the high-K^+, Na^+-free pipette solution with 1 mM ATP and 5 mM P_i. Following that change of external solution, strophanthidin-sensitive currents were zero at all times and all potentials (Fig. 3*C c–d*), showing that K^+ translocation by the Na^+/K^+ pump does not involve net charge movement within the membrane field. The same result was obtained in experiments in which the pipette ATP concentration was 5 mM.

DISCUSSION

The complete absence of strophanthidin-sensitive current under conditions appropriate for K^+/K^+ exchange (Fig. 3*C*) provides direct evidence that K^+ transport by the Na^+/K^+ pump does not include a charge-translocating, and hence voltage-sensitive, step. It is unlikely that very fast transient pump currents did occur and that we failed to detect them. Membrane current was faithfully recorded at all membrane potentials within 1 ms of the start of the voltage step, so that any transient with a time constant ≥1 ms (rate constant, ≤ 1000 s^{-1}) should have been detected. Even if the hypothetical voltage-sensitive step were faster than that (for example, reflecting K^+ concentration changes in an "ion well" within the membrane field), decay of any resulting net charge movement should be governed by the rate-limiting step (e.g., refs. 25, 26), which, for K^+ translocation, is probably much slower than 1000 s^{-1}, as discussed below. We therefore conclude that K^+ translocation does not involve charge movement and thus is voltage insensitive. The same conclusion is suggested by experiments on partially purified Na^+/K^+ pumps reconstituted into artificial phospholipid vesicles, in which a potential set with ionophores did not alter the rates of Rb^+/Rb^+ exchange, or of Na^+/K^+ transport at low ATP concentration (when K^+ translocation would be expected to be rate limiting) (6), or the rates of conformational changes of K^+-loaded, fluorescein-labeled enzyme (12).

Over a relatively wide range of Na^+/K^+ pump rates at various $[K^+]_o$ (Fig. 1*B*) and at various internal Na^+ concentrations $[Na^+]_i$ and $[Na^+]_o$ (27), we find no consistent evidence for a negative-slope region in the pump I–V relationship between −140 and +60 mV (the occasional single point notwithstanding—e.g., Fig. 1*B*, 1 mM $[K^+]_o$): steady-state pump current seems to approach a saturating, voltage-independent, outward level when the pump cycle runs forward (Fig. 1*B*) and a saturating inward level when the cycle runs backward (Fig. 2*C*). Recent experiments on squid giant axons (24) using improved techniques to minimize contaminating K^+ currents and electrical end-effects, yielded monotonic I–V relationships for the backward-running Na^+/K^+ pump closely similar to that in Fig. 2*D*, suggesting that the negative slope inferred from earlier

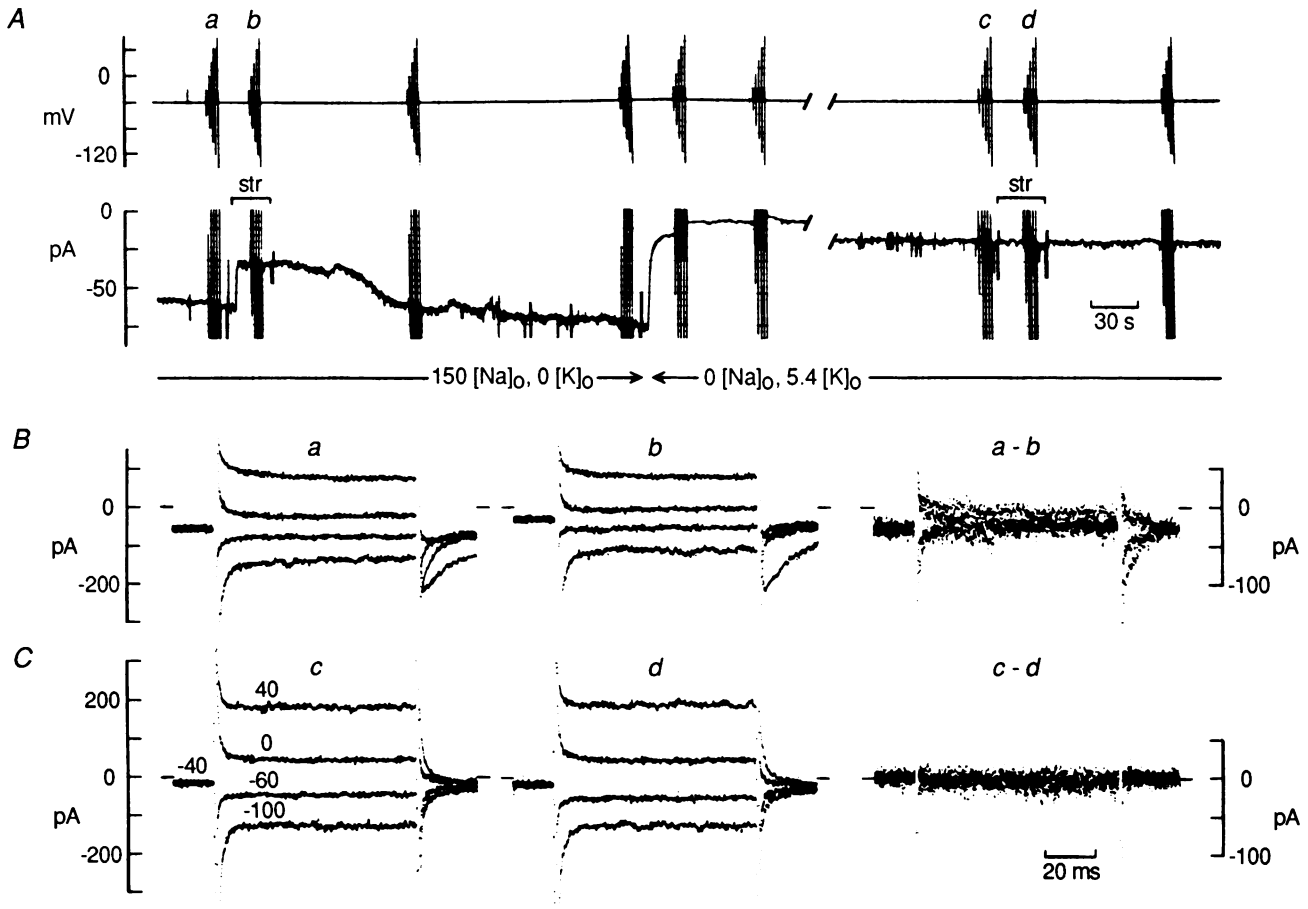
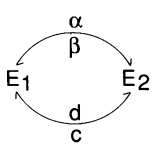


FIG. 3. Absence of strophanthidin-sensitive current during K^+/K^+ exchange. (*A*) Chart recording of membrane potential (*Upper*) and membrane current (*Lower*); holding potential, −40 mV. The gap marks omission of 9 min of record; *a–d* denote acquisition of the I–V data represented in *B* and *C*. Bars above the current record indicate exposures to 2 mM strophanthidin (str), and arrows below signify the switch from a K^+-free, 150 mM Na^+ external solution, identical to that of Fig. 2, to one containing 5.4 mM K^+ and 145 mM *N*-methyl-D-glucamine$^+$ in place of Na^+. Internal solution was the same as in Fig. 2 except ATP concentration was 1 mM. (*B*) Superimposed current traces for 80-ms voltage pulses to +40, 0, −60, and −100 mV from *A*, recorded during backward pumping (*a*) or in the presence of strophanthidin (*b*). The high-gain, strophanthidin-sensitive currents (*a* − *b*) were obtained by subtracting records in *b* from counterparts in *a*. (*C*) Superimposed current records as in *B*, but recorded during K^+/K^+ exchange without (*c*) or with (*d*) strophanthidin. The high-gain strophanthidin-sensitive currents (*c* − *d*) were obtained by appropriate subtraction. Total cell capacitance, 177 pF. Initial pipette resistance, 1.1 MΩ; 1.3 MΩ of the 2.5 MΩ series resistance was compensated.

measurements (23) was artifactual. The apparent negative slope in the forward pump I–V relationship of *Xenopus* oocytes at membrane potentials >20 mV (9) might also be attributable to contamination (1, 28), in that case by a $[K]_o$-sensitive, voltage-dependent current (evident in figure 12 of ref. 9).

The monotonic voltage dependence of both outward (Fig. 1*B*) and inward (Fig. 2*C*) steady Na^+/K^+ pump current implies that net charge moves in only one direction through the membrane field during each pump cycle (10, 11, 20). Since present evidence suggests that Na^+ translocation is voltage-sensitive (3, 4, 6, 25, 26) because it involves motion of a single positive charge (3), sufficient for a complete 3 Na^+/2 K^+ transport cycle, the simplest interpretation of all these findings is that this single-charge translocation constitutes the only voltage-dependent step in the pump cycle (cf. refs. 3, 6). Hansen *et al.* (29) showed that any multi-step cycle with a *single* voltage-sensitive step may be represented by a reduced, pseudo-two-state kinetic scheme (21, 30):



where empirical voltage-dependent rate constants α and β are instantaneous functions of membrane potential, α increasing and β decreasing for positive voltage jumps, and c and d are empirical, lumped rate constants representing all voltage-independent steps in the original cycle. If voltage-independent steps were rate limiting—i.e., $c + d < \alpha + \beta$—steady-state concentrations of enzyme intermediates E_1 and E_2 ($[E_1]$, $[E_2]$) would be determined largely by α and β and would therefore be voltage dependent. A positive voltage step would thus elicit an initial outward current jump due to the instantaneous increase in α and decrease in β, followed by an exponential relaxation, with a time constant $(\alpha + \beta + c + d)^{-1}$, due to a fall in $[E_1]$ and a rise in $[E_2]$ towards their new steady levels; the ratio of the amplitudes of the transient-to-steady current changes is given by $(\alpha + \beta)/(c + d)$ (21). If, on the other hand, the voltage-dependent step were severely rate limiting ($\alpha + \beta << c + d$), there would be almost no transient current because $[E_1]$ and $[E_2]$ would be controlled by voltage-independent rate constants and so would not change after a voltage jump; in that case, the new steady level of pump current would be established instantaneously and, under appropriate ("zero-trans," i.e., one-way transport) conditions, its voltage dependence would reveal directly the voltage dependence of α and β.

Accordingly, the large transient pump currents in Figs. 1–3 (large relative to the steady current changes) provide clear

evidence that, under the conditions of these experiments, the rate-limiting steps in both forward and reverse pump cycles are voltage independent. Our second conclusion, then, is that the overall rate of the pump cycle is voltage dependent not because the voltage-sensitive step is rate limiting, but because it controls the *concentration* of the enzyme intermediate that enters the rate-limiting step ($[E_2]$ for the forward cycle and $[E_1]$ for the backward cycle). The $[K^+]_o$ dependence of both the steady amplitude of forward pump current and the decay rate of the transient component, (Fig. 1) suggests that this rate-limiting step in the cycle reflects K^+ translocation. Thus, assuming that a single charge moves per pump (3), the maximum quantity, 4.2 pC, of mobile charge in this cell at 0 mM $[K^+]_o$ gives a total of 26×10^6 pumps per cell, or a density, related to linear membrane capacitance (218 pF for this cell) of 12×10^4 pF^{-1}, so that the saturating steady-state outward current at positive potentials (Fig. 1*B*) then yields estimates (29) of the voltage-independent forward rate constant c of 1, 24, and 55 s^{-1} at 0, 1, and 5.4 mM $[K^+]_o$, respectively. The decay rates of the transient pump currents in Fig. 1*Aa* and the ratios of the transient-to-steady current amplitudes suggest (via the above relationships) that at 0 mM $[K^+]_o$ both voltage-independent rate constants are negligibly small and, hence, that the sum $\alpha + \beta$ is $\approx$180 s^{-1} at positive potentials (cf. ref. 3). The evident, incremental speeding of the transients at 1 and 5.4 mM $[K^+]_o$ (Fig. 1*A*) is then at least qualitatively consistent with the $[K^+]_o$-dependent increases of the voltage-insensitive forward rate constant c estimated above from the steady currents. [Quantitative differences are not unexpected because the lumping of voltage-independent rate constants is strictly applicable only in the steady state (21, 29).]

It will be interesting to see whether the rates of other electrogenic ion transporters are similarly limited by voltage-independent steps, or whether, instead, they are limited by a charge-translocating step. Considering Na^+/Ca^{2+} exchange (19), for example, should monotonic inward and outward I–V relations be taken to signify the presence of a single charge-translocating step in the Na^+/Ca^{2+} transport cycle, the apparent lack of current relaxations after voltage jumps (19) might indicate that the transport rate of the ion exchanger is strongly limited by that charge-translocating step—in stark contrast to the simplified scheme described here for the Na^+/K^+ pump.

We thank Dr. Paul F. Cranefield for constant encouragement and Diane Schenkman and Peter Hoff for technical assistance. This work was supported by grants from the National Institutes of Health (HL-14899 and HL-36783) and the American Heart Association New York City Affiliate. D.C.G. is an Irma T. Hirschl Career Scientist.

1. De Weer, P., Gadsby, D. C. & Rakowski, R. F. (1988) *Annu. Rev. Physiol.* **50,** 225–241.
2. Fendler, K., Grell, E., Haubs, M. & Bamberg, E. (1985) *EMBO J.* **4,** 3079–3085.
3. Nakao, M. & Gadsby, D. C. (1986) *Nature (London)* **323,** 628–630.
4. Borlinghaus, R., Apell, H. J. & Läuger, P. (1987) *J. Membr. Biol.* **97,** 161–178.
5. Rephaeli, A., Richards, D. E. & Karlish, S. J. D. (1986) *J. Biol. Chem.* **261,** 12437–12440.
6. Goldshlegger, R., Karlish, S. J. D., Rephaeli, A. & Stein, W. D. (1987) *J. Physiol. (London)* **387,** 331–355.
7. Forbush, B. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 5310–5314.
8. Karlish, S. J. D. & Kaplan, J. H. (1985) in *The Sodium Pump: Fourth International Conference on Na,K ATPase*, eds. Glynn, I. M. & Ellory, C. (Company of Biologists, Cambridge, UK), pp. 501–506.
9. Lafaire, A. V. & Schwarz, W. (1986) *J. Membr. Biol.* **91,** 43–51.
10. Reynolds, J. A., Johnson, E. A. & Tanford, C. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 6869–6873.
11. Läuger, P. & Apell, H.-J. (1986) *Eur. Biophys. J.* **13,** 309–321.
12. Rephaeli, A., Richards, D. E. & Karlish, S. J. D. (1986) *J. Biol. Chem.* **261,** 6248–6254.
13. Gadsby, D. C., Kimura, J. & Noma, A. (1985) *Nature (London)* **315,** 63–65.
14. Hamill, O. P., Marty, A., Neher, E., Sakmann, B. & Sigworth, F. J. (1981) *Pflügers Arch.* **391,** 85–100.
15. Soejima, M. & Noma, A. (1984) *Pflügers Arch.* **400,** 424–431.
16. Bahinski, A., Gadsby, D. C. & Nakao, M. (1988) *J. Physiol. (London)* **396,** 52P (abstr.).
17. Isenberg, G. & Klöckner, U. (1982) *Pflügers Arch.* **395,** 6–18.
18. Glynn, I. M. & Karlish, S. J. D. (1975) *Annu. Rev. Physiol.* **37,** 13–55.
19. Kimura, J., Miyamae, S. & Noma, A. (1987) *J. Physiol. (London)* **384,** 199–222.
20. Chapman, J. B., Johnson, E. A. & Kootsey, J. M. (1983) *J. Membr. Biol.* **74,** 139–153.
21. Hansen, U.-P., Tittor, J. & Gradmann, D. (1983) *J. Membr. Biol.* **75,** 141–169.
22. Garrahan, P. J. & Glynn, I. M. (1967) *J. Physiol. (London)* **192,** 237–256.
23. De Weer, P. & Rakowski, R. F. (1984) *Nature (London)* **309,** 450–452.
24. Rakowski, R. F., De Weer, P. & Gadsby, D. C. (1988) *Biophys. J.* **53,** 223a (abstr.).
25. Apell, H. J., Borlinghaus, R. & Läuger, P. (1987) *J. Membr. Biol.* **97,** 179–191.
26. Fendler, K., Grell, E. & Bamberg, E. (1987) *FEBS Lett.* **224,** 83–88.
27. Gadsby, D. C. & Nakao, M. (1987) *J. Physiol. (London)* **382,** 106P (abstr.).
28. Rakowski, R. F. (1987) *J. Gen. Physiol.* **90,** 34a–35a (abstr.).
29. Hansen, U.-P., Gradmann, D., Sanders, D. & Slayman, C. L. (1981) *J. Membr. Biol.* **63,** 165–190.
30. De Weer, P. (1984) in *Electrogenic Transport: Fundamental Principles and Physiological Implications*, eds. Blaustein, M. P. & Lieberman, M. (Raven, New York), pp. 1–15.