SI Appendix

Simplified Kinetic Scheme of Na/K Pump Function. ATP-dependent K_i^+ deocclusion, Na_i^+ binding, and phosphorylation were lumped together in a single reaction rate, ρ (with the plausible assumption that the reverse reactions will not occur under our experimental conditions) similarly to Sagar and Rakowski (1). The differential equations describing the model are:

$$\frac{dE1P(Na_3)}{dt} = \rho \cdot E2(K_2) + \beta_3 \cdot E2P(Na_2) - \alpha_3 \cdot E1P(Na_3)$$

$$\frac{dE2P(Na2)}{dt} = \alpha_3 \cdot E1P(Na_3) + \beta_2 \cdot E2P - \beta_3 \cdot E2P(Na_2) - \alpha_2 \cdot E2P(Na_2)$$

$$\frac{dE2P}{dt} = \alpha_2 \cdot E2P(Na_2) + \beta_1 \cdot E2(K_2) - \beta_2 \cdot E2P - \alpha_1 E2P$$

$$\frac{dE2(K_2)}{dt} = \alpha_1 E2P - \rho \cdot E2(K_2) - \beta_1 \cdot E2(K_2)$$

and the conservation equation:

$$E1P(Na_3) + E2P(Na_2) + E2P + E2(K_2) = 1$$

where $E1P(Na_3)$, $E2P$, etc. depict the fractional occupancy of each state. Analogous to Heyse et al. (2) we describe the release of the first Na^+ ion as the most voltage-dependent pseudo first order reaction rate $\beta_3 = \beta_3^0 \cdot [Na]_o \cdot \exp(-\lambda_3 \cdot \frac{F}{RT} \cdot V)$, which combines Na^+ binding to its exclusive site, Na^+ re-occlusion, and the reverse conformational transition. F, R and T have their usual meaning, V is the transmembrane voltage and λ_3 is the fraction of the electric field traveled by the Na^+ ion to its binding site (0.75; refs. 1, 2). The general model including the voltage-dependent binding of the two other Na^+ ions (in the reverse reaction) or the two K^+ ions (in the forward reaction) will include similar expressions for β_2 and α_1, but with smaller λ (0.1-0.4). For simplicity, to illustrate the effect of modifying Na^+ binding to the shared sites and to obtain an explicit expression for the centers ($V_{½}$) of the I_P-V curve and the Q-V curve as a function of the other rates, we consider the case in which ion binding to the shared sites is not voltage-dependent (i.e. $\beta_2 = \beta_2^0[Na]_o$ and $\alpha_1 = \alpha_1^0[K]_o$).

Similar to the description by (4), the steady state pump current (I_P) at any given time will be given by $I_P = \rho \cdot E2(K_2)$, in which

$$E2(K_2) = \frac{\alpha_1\alpha_2\alpha_3}{\alpha_1\alpha_2\alpha_3 + \alpha_2\alpha_3\rho + \alpha_2\alpha_3\beta_1 + \alpha_1\alpha_2\rho + \alpha_3\beta_2\rho + \beta_2\beta_3\rho + \beta_1\beta_2\beta_3 + \alpha 3\beta_1\beta_2 + \alpha_1\alpha_3\rho + \alpha_1\beta_3\rho} \quad \textbf{[1]}$$

In the absence of K_o^+ $\alpha_1 = 0$ and the kinetic scheme is reduced to the top line describing the transition between $E1P(Na_3)$ and E2P. The steady state distribution of the slow component of charge movement associated with the release of the first Na^+ ion is given by the steady state occupancy of $E1P(Na_3)$:

$$E1(Na_3) = \frac{\beta_2\beta_3}{\alpha_2\alpha_3 + \alpha_3\beta_2 + \beta_2\beta_3} \quad \textbf{[2]}$$

In order to find the center of the I_P-V curve in the presence of K^+_o ($V_{½IP}$) and of the Q-V curve in the absence of K^+_o ($V_{½Q}$) we equated both $E2(K_2)$ and $E1(Na_3)$, respectively, to 0.5 and solved for the rate constant for Na^+ binding to its exclusive site, β_3 (and therefore for $V_{1/2}$) as follows:

$$V_{½IP} = -0.033 * \ln\left[\frac{\alpha_1\alpha_2\alpha_3 - \alpha_2\alpha_3\rho - \alpha_2\alpha_3\beta_1 - \alpha_1\alpha_2\rho - \alpha_1\alpha_3\rho - \beta_2(\alpha_3\rho + \alpha_3\beta_1)}{\beta_3^0\,[Na]_o\{\beta_2(\rho + \beta_1) + \alpha_1\rho\}}\right] \quad \textbf{[3]}$$

$$V_{½Q} = -0.033[\ln\alpha_3 + \ln(\alpha_2 + \beta_2) - \ln(\beta_3^0\,[Na]_o) - \ln\beta_2 \quad \textbf{[4]}$$

Let's first note that, from Eqs. **3** and **4**, if the ΔKESYY mutation affected Na^+ binding to the exclusive binding site (β_3^0) a large shift of identical magnitude would occur in both I_P- and Q-V curves. On the other hand, if β_3^0 is not modified by the mutation and only β_2 is affected, as we propose, β_3^0 cancels out when subtracting $V_{½IP}^{\Delta KESYY} - V_{½IP}^{control}$ and will not contribute to the magnitude of the shifts.

For simplicity we begin discussing the more straight forward effects on the Q-V. Assuming that the only effect of the ΔKESYY deletion is a 16-fold reduction in the rate of Na^+ binding to the shared sites (i.e. a reduction in β_2^0), we can calculate the shift induced in the Q-V curve from Eq. (4) as the difference:

$$V_{½Q}^{\Delta KESYY} - V_{½Q}^{control} = -0.033 * \{\ln[(16\alpha_2 + \beta_2)/16(\alpha_2 + \beta_2)] - \ln(1/16)\}$$

Although we cannot calculate values for α_2 and β_2 in our preparation, according to Heyse *et al*. (ref. 2; values in table V) at a $[Na^+_o]$ = 125 mM $\beta_2 << \alpha_2$. Thus, in our experiments the ratio of the first logarithmic term is ≈ 1, which gives

$$V_{½Q}^{\Delta KESYY} - V_{½Q}^{control} \cong 0.033 * \ln(1/16) = -91 \text{ mV}$$

This value is almost identical to our observed values (-91 mV in TEVC and -94 mV in patch clamp data).

The effect of a reduction in β_2 in the center of the I_P-V curve is far less obvious and we need to use other estimates for the discussion. We estimate the maximum turnover rate, ρ, at ~15 s^{-1} (I_{Pmax}/Q_{max}, see Fig. 2 legend), whereas the rate of transient charge movement at positive potentials yields an estimate of 200 $s^{-}1$ for α_3 (we use TEVC data because intracellular conditions are the same as those used for I_p measurements).

Again, based on Heyse et al. (2), $\alpha_2 \sim 1*10^4$ s^{-1} and $\beta_2^0 \sim 2*10^3$ $M^{-1}s^{-1}$ and $\beta_1 \sim$ 10 s^{-1}. Thus, for a K_o affinity of 100 μM, $\alpha_1^0 \sim 10^4$ $M^{-1}s^{-1}$. Using 10 mM K^+_o for an initial calculation it is clear that any effect on β_2 will be insignificant in the numerator because $\alpha_1\alpha_2\alpha_3 \sim 10^8$, whereas the rest of the terms combined reach a maximum of $\sim 3*10^7$ and the terms with β_2 are on the order of 10^6 for RD control pumps.

In the denominator the results will depend on the actual values of α_1 and β_2 (which depend on $[Na^+]$ and $[K^+]$). However, it is clear that if $[K^+]_o$ is high enough (i.e. near-saturating K^+_o) $\alpha_1 \geq \beta_2$, even in control pumps and thus, no significant shift will be produced. Consequently, this model predicts a lack of effect of mutants that alter binding of extracellular Na^+ to the shared sites on the I_P-V curve.

1. Sagar A, Rakowski RF (1994) Access channel model for the voltage dependence of the forward-running Na+/K+ pump. *J Gen Physiol* 103:869-893.
2. Heyse S, Wuddel I, Apell HJ, Sturmer W (1994) Partial reactions of the Na,K-ATPase: Determination of rate constants. *J Gen Physiol* 104:197-240.