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Supplementary Information

Calculation of the steady-state pump current

Based on the Albers-Post model of the Na^+,K^+-ATPase enzymatic cycle shown in Fig. 1, the differential rate equations describing the changes in concentrations of all the enzyme intermediates are:

$$\frac{d[E1Na_3^+]}{dt} = -k_1[E1Na_3^+] - k_{dN1}^{i}[E1Na_3^+] + k_{bN1}^{i}[E1Na_2^+][Na^+]_i \quad \text{(S1)}$$

$$\frac{d[E1P(Na^+)_3]}{dt} = k_1[E1Na_3^+] - k_{2f}[E1P(Na^+)_3] + k_{2r}[E2PNa^+_3] \quad \text{(S2)}$$

$$\frac{d[E2PNa_3^+]}{dt} = k_{2f}[E1P(Na^+)_3] - k_{2r}[E2PNa_3^+] - k_{dN1}^{o}[E2PNa_3^+] + k_{bN1}^{o}[E2PNa_2^+][Na^+]_o \quad \text{(S3)}$$

$$\frac{d[E2PNa_2^+]}{dt} = k_{dN1}^{o}[E2PNa_3^+] - k_{bN1}^{o}[E2PNa_2^+][Na^+]_o - k_{31}[E2PNa_2^+] - 2k_{dN}^{o}[E2PNa_2^+] + k_{bN}^{o}[E2PNa^+][Na^+]_o \quad \text{(S4)}$$

$$\frac{d[E2PNa^+]}{dt} = 2k_{dN}^{o}[E2PNa_2^+] - k_{bN}^{o}[E2PNa^+][Na^+]_o - k_{bK}^{o}[E2PNa^+][K^+]_o + k_{dK}^{o}[E2PNa^+K^+] - k_{dN}^{o}[E2PNa^+] + 2k_{bN}^{o}[E2P][Na]_o \quad \text{(S5)}$$

$$\frac{d[E2P]}{dt} = k_{dN}^{o}[E2PNa^+] - 2k_{bN}^{o}[E2P][Na^+]_o - 2k_{bK}^{o}[E2P][K^+]_o + k_{dK}^{o}[E2PK^+] \quad \text{(S6)}$$

$$\frac{d[E2PK^+]}{dt} = 2k_{bK}^{o}[E2P][K^+]_o - k_{dK}^{o}[E2PK^+] - k_{bN}^{o}[E2PK^+][Na^+]_o + k_{dN}^{o}[E2PK^+Na^+] - k_{bK}^{o}[E2PK^+][K^+]_o + 2k_{dK}^{o}[E2PK_2^+] \quad \text{(S7)}$$

$$\frac{d[E2PK_2^+]}{dt} = k_{bK}^{o}[E2PK^+][K^+]_o - 2k_{dK}^{o}[E2PK_2^+] - k_{32}[E2PK_2^+] \quad \text{(S8)}$$

$$\frac{d[E2(K^+)_2]}{dt} = k_{32}[E2PK_2^+] + k_{4r}[E1K^+_2] - k_{4f}[E2(K^+)_2] \quad \text{(S9)}$$

$$\frac{d[E1K^+_2]}{dt} = k_{4f}[E2(K^+)_2] - k_{4r}[E1K_2^+] - 2k_{dK}^i[E1K_2^+] + k_{bK}^i[E1K^+][K^+]_i \quad \text{(S10)}$$

$$\frac{d[E1K^+]}{dt} = 2k_{dK}^i[E1K_2^+] - k_{bK}^i[E1K^+][K^+]_i + k_{dN}^i[E1K^+Na^+] - k_{bN}^i[E1K^+][Na^+]_i + 2k_{bK}^i[E1][K^+]_i - k_{dK}^i[E1K^+] \quad \text{(S11)}$$

$$\frac{d[E1]}{dt} = k_{dK}^i[E1K^+] - 2k_{bK}^i[E1][K^+]_i + k_{dN}^i[E1Na^+] - 2k_{bN}^i[E1][Na^+]_i \quad \text{(S12)}$$

$$\frac{d[E1Na^+]}{dt} = 2k_{bN}^i[E1][Na^+]_i - k_{dN}^i[E1Na^+] + k_{dK}^i[E1Na^+K^+] - k_{bK}^i[E1Na^+][K^+]_i + 2k_{dN}^i[E1Na_2^+] - k_{bN}^i[E1Na^+][Na^+]_i \quad \text{(S13)}$$

$$\frac{d[E1Na_2^+]}{dt} = k_{bN}^i[E1Na^+][Na^+]_i - 2k_{dN}^i[E1Na_2^+] + k_{4f}[E2(Na^+)_2] + k_{dN1}^i[E1Na_3^+] - k_{bN1}^i[E1Na_2^+][Na^+]_i \quad \text{(S14)}$$

$$\frac{d[E2(Na^+)_2]}{dt} = k_{31}[E2PNa_2^+] - k_{4f}[E2(Na^+)_2] \quad \text{(S15)}$$

$$\frac{d[E2PNa^+K^+]}{dt} = k_{bK}^o[E2PNa^+][K^+]_o - k_{dK}^o[E2PNa^+K^+] \quad \text{(S16)}$$

$$\frac{d[E1Na^+K^+]}{dt} = k_{bK}^i[E1Na^+][K^+]_i - k_{dK}^o[E1Na^+K^+] \quad \text{(S17)}$$

$$\frac{d[E2PK^+Na^+]}{dt} = k_{bN}^o[E2PK^+][Na^+]_o - k_{dN}^o[E2PK^+Na^+] \quad \text{(S18)}$$

$$\frac{d[E1K^+Na^+]}{dt} = k_{bN}^i[E1K^+][Na^+]_i - k_{dN}^o[E1K^+Na^+] \quad \text{(S19)}$$

The effects of the transmembrane electrical potential difference V (defined as electrical potential in minus electrical potential out) are taken into account by the following Boltzmann expressions:

$$k_{bN1}^i = k_{bN1,V=0}^i \exp(0.25FV/RT) \quad \text{(S20)}$$

$$k_{dN1}^i = k_{dN1,V=0}^i \exp(-0.25FV/RT) \quad \text{(S21)}$$

$$k_{2f} = k_{2f,V=0} \exp(0.1FV/RT) \quad \text{(S22)}$$

$$k_{2r} = k_{2r,V=0} \exp(-0.1FV / RT) \quad \text{(S23)}$$

$$k^{o}_{bN1} = k^{o}_{bN1,V=0} \exp(-0.65FV / RT) \quad \text{(S24)}$$

$$k^{o}_{dN1} = k^{o}_{dN1,V=0} \exp(0.65FV / RT) \quad \text{(S25)}$$

$$k^{o}_{bN} = k^{o}_{bN,V=0} \exp(-0.185FV / RT) \quad \text{(S26)}$$

$$k^{o}_{dN} = k^{o}_{dN,V=0} \exp(0.185FV / RT) \quad \text{(S27)}$$

$$k^{o}_{bK} = k^{o}_{bK,V=0} \exp(-0.185FV / RT) \quad \text{(S28)}$$

$$k^{o}_{dK} = k^{o}_{dK,V=0} \exp(0.185FV / RT) \quad \text{(S29)}$$

The fluxes through the $3Na^+/2Na^+$ and $3Na^+/2K^+$ exchange pathways, C1 and C2 respectively, are given by:

$$C1 = k_{31}[E2PNa_2^+] \quad \text{(S30)}$$

$$C2 = k_{32}[E2PK_2^+] \quad \text{(S31)}$$

The values of all the rate constants used for the simulations are listed in Table S1.

TABLE S1 Values of the rate constants for the reaction scheme shown in Fig. 1.

Parameter	Value	Reference
k_1	200 s^{-1}	47
$k_{2f,V=0}$	225 s^{-1}	48
$k_{2r,V=0}$	401 s^{-1}	48
$k^o_{dN1,V=0}$	10^5 s^{-1} [a]	17
$k^o_{bN1,V=0}$	1000 M^{-1} s^{-1} [b]	17, 35
$k^o_{dN,V=0}$	3000 s^{-1}	17
$k^o_{bN,V=0}$	7.5 mM^{-1} s^{-1} [c]	17, 48
$k^o_{dK,V=0}$	3000 s^{-1} [d]	-
$k^o_{bK,V=0}$	2260 mM^{-1} s^{-1} [e]	20
k_{31}	4 s^{-1}	20, 49
k_{32}	342 s^{-1}	21
k_{4f}	90 s^{-1}	50
k_{4r}	550 s^{-1}	51
$k^i_{dN1,V=0}$	3000 s^{-1} [f]	-
$k^i_{bN1,V=0}$	1670 M^{-1} s^{-1} [g]	47
k^i_{dN}	3000 s^{-1} [h]	-
k^i_{bN}	375 mM^{-1} s^{-1} [i]	47
k^i_{dK}	3000 s^{-1} [j]	-
k^i_{bK}	300 mM^{-1} s^{-1} [k]	18

[a] This value has been estimated from [17] as a lower limit, because the kinetics of this reaction could not be resolved by the voltage-jump technique.

[b] This value has been obtained by dividing the value of $k^{o}_{dN1,V=0}$ by the dissociation constant for this reaction of 100 mM [35].

[c] This value has been obtained by dividing the value of $k^{o}_{dN,V=0}$ by the dissociation constant for this reaction of 400 mM [48].

[d] This value has been estimated based on the assumption that the rate constant for extracellular dissociation of K^+ is approximately equal to that of the second and third Na^+ ions [17].

[e] This value has been obtained by dividing the value of $k^{o}_{dK,V=0}$ by the dissociation constant for this reaction of 1.33 mM [20].

[f] This value has been estimated based on the assumption that the rate constant for cytoplasmic dissociation of Na^+ from its specific site is approximately equal to that of the second and third extracellular Na^+ ions [17].

[g] This value has been obtained by dividing the value of $k^{i}_{dN1,V=0}$ by the dissociation constant for this reaction of 1.8 mM [47].

[h] This value has been estimated based on the assumption that the rate constant for cytoplasmic dissociation of Na^+ from its nonspecific site is approximately equal to that of the second and third extracellular Na^+ ions [17].

[i] This value has been obtained by dividing the value of k^{i}_{dN} by the dissociation constant for this reaction of 8 mM [47].

[j] This value has been estimated based on the assumption that the rate constant for cytoplasmic dissociation of K^+ from its nonspecific site is approximately equal to that of the second and third extracellular Na^+ ions [17].

[k] This value has been obtained by dividing the value of k^{i}_{dK} by the dissociation constant for this reaction of 10 mM [18].

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