Supplemental material

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Description of simulations

All simulations were performed in MATLAB. Equation sets employed were as follows.

NCX simulations

Exchangers are assumed to exist in four states (F_1–F_4). F_1 is the inactive state with a bound regulatory Ca and closed PIP_2 gate. F_2 is the active state. F_3 is the inactive state with a bound Ca and open PIP_2 gate. F_4 is the inactive state without regulatory Ca and closed PIP_2 gate. Full occupation of three Na transport sites (F_{3n}) is assumed to be proportional to a Hill equation with a slope of 2.5 and a K_{50} for Na (N_i) of 17 mM. Both outward exchange current (Incx) transport and inactivation via the PIP_2 gate are proportional to F_{3n}:

$$F_{3n} = Ni^{2.5}/(Ni^{2.5}+17^{2.5}), \quad (1)$$

$$dF_1/dt = F_4 \times C_i \times K_{con1}\text{-}F_1 \times K_{coff1}+F_2 \times F_{3n} \times K_{inact1}\text{-}F_1 \times K_{12}, \quad (2)$$

$$dF_2/dt = F_3 \times C_i \times K_{con2}\text{-}F_2 \times K_{con2}+F_1 \times K_{12}\text{-}F_2 \times F_{3n} \times K_{inact1}, \quad (3)$$

$$dF_3/dt = F_2 \times K_{coff2}+F_4 \times K_{43}\text{-}F_3 \times C_i \times K_{con2}\text{-}F_3 \times F_{3n} \times K_{inact2}, \quad (4)$$

$$F_4 = 1\text{-}F_1\text{-}F_2\text{-}F_3, \quad (5)$$

$$I_{ncx} = F_2 \times F_{3n}. \quad (6)$$

Parameter settings for Fig. 2

K_{con1} = 0.1 $\mu M^{-1}s^{-1}$; K_{coff1} = 0.05 $\mu M^{-1}s^{-1}$; K_{con2} = 20 s^{-1}; K_{coff2} = 0.3 s^{-1}; K_{inact1} = 0.2 s^{-1}; K_{inact2} = K_{inact1} × 25 s^{-1}; K_{12} = 0.2 s^{-1}; K_{43} = 0.1 s^{-1}. Note that the two Na-dependent inactivation reactions are assumed to both be regulated by anionic phospholipids in the simulation presented.

Na/K pump simulations

The minimal Na/K pump model employed assumes that two Na and two K bind competitively in both E_1 and E_2 configurations and that a third Na ion binds independently at a separate site. 90% of voltage dependence is partitioned to extracellular Na binding to the Na-selective site, and 10% is partitioned to reactions that translocate three Na. Na translocation rates are simulated to be twice faster than K translocation. The simulated Na/K pump activity reproduces the concentration and voltage dependencies of Na/K pumps in reasonable approximation, and the model is consistent with a free energy of ATP hydrolysis of 52 kJ/mol under the conditions simulated. Inactivation occurs from all E_1 Na sites that are not fully occupied, and recovery occurs from fully Na occupied E_1 sites.

F_{no}, F_{ni}, F_{n2o}, F_{n2i}, F_{k2O}, and F_{k2i} give the fractional occupation of ion-binding sites; F_{ATP}, F_{pi} and F_{ADP} give the fractional occupation of nucleotide and phosphate sites; and D_i, Do, K_1, K_2, K3, and K_4 are temporary variables. Na and K binding are mutually exclusive, as are ATP and P_i binding. E_m is in millivolts. N_o, K_o, N_i, and K_i are the extracellular and intracellular Na and K concentrations, and the 10 ion dissociation constants are designated with parameter settings.

$$F_{no} = N_o/(N_o+K_{no3} \times e^{Em/26\times0.9}), \quad (7)$$

$$D_o = 1 + N_o/K_{no1} \times (1 + No/K_{no2})+K_o/K_{ko1} \times (1 + K_o/K_{ko2}), \quad (8)$$

$$F_{k2o} = K_o \times K_o/K_{ko1}/K_{ko2}/D_o, \quad (9)$$

$$F_{n2o} = N_o \times N_o/K_{no1}/K_{no2}/D_o, \quad (10)$$

$$F_{ni} = N_i/(N_i+K_{ni3}), \quad (11)$$

$$D_i = 1 + N_i/K_{ni1} \times (1 + N_i/K_{ni2})+K_i/K_{ki1} \times (1 + K_i/K_{ki2}), \quad (12)$$

$$F_{k2i} = K_i \times K_i/K_{ki1}/K_{ki2}/D_i, \quad (13)$$

$$F_{n2i} = N_i \times N_i/K_{ni1}/K_{ni2}/D_i, \quad (14)$$

$$F_{ADP} = ADP/(ADP + K_{ADP}), \quad (15)$$

$$F_{ATP} = ATP/K_{ATP}/(1 + ATP/K_{ATP}+P_i/K_{pi}), \quad (16)$$

$$F_{pi} = P_i/K_{pi}/(1 + ATP/K_{ATP}+P_i/K_{pi}), \quad (17)$$

$$K_1 = 2 \times F_{ni} \times F_{n2i} \times F_{ATP} \times (1\text{-}F_{ADP}) \times e^{Em/55\times0.1}, \quad (18)$$

$$K_2 = 2 \times F_{no} \times F_{n2o} \times (F_{ADP}+0.32) \times e^{-Em/55\times0.1}, \quad (19)$$

$$K_3 = F_{k2o} \times (1\text{-}F_{no}), \quad (20)$$

$K_4 = F_{k2i} \times F_{pi} \times (1\text{-}F_{ni})$, (21)

$E_2 = (K_1+K_4)/(K_1+K_2+K_3+K_4)$, (22)

$E_1 = 1\text{-}E_2$. (23)

From the E_1 and E_2 fractions, a fractional pump activity, Pump, was calculated:

$Pump = E_1 \times K_1\text{-}E_2 \times K_2$. (24)

Inactivation was simulated as the fraction of pumps in the active state (F_{act}):

$dF_{act}/dt = (1\text{-}F_{act}) \times F_{n2i} \times F_{ni} \times K_{rec}\text{-}E_1 \times F_{act} \times ((1\text{-}F_{n2i})+(1\text{-}F_{ni})) \times K_{inact}$. (25)

The pump current is then

$I_{pump} = Pump \times F_{act} \times K_{pump}$. (26)

For results presented in Fig. 6, the cytoplasmic Na concentration (N_i) was simulated assuming a constant background Na current (I_{na}), a 10-pl cytoplasmic mixing volume, and an exchange time constant between the pipette and cytoplasm, tau_{pip}, of 12 s.

With the pipette Na concentration being Na_{pip}, cytoplasmic Na is simulated as:

$dN_i/dt = (I_{na}\text{-}I_{pump} \times 3)/1,000 + (Na_{pip}\text{-}N_i)/tau_{pip}$. (27)

Parameter settings for Fig. 6

Na_{pip} = 20 mM; N_o = 120 mM; K_o = 7.0 mM; K_i = 120 mM; ADP = 0.05 mM; P_i = 0.3 mM; ATP = 6 mM; Na_{pip} = 20 mM; E_m = 0 mV; tau_{pip} = 12 s; K_{rec} = 0.8 s^{-1}; K_{pump} = 2.5 nA; K_{no3} = 900 mM; K_{no1} = 10 mM; K_{no2} = 10 mM; K_{ko1} = 0.3 mM; K_{ko2} = 0.2 mM; K_{ni3} = 4 mM; K_{ni1} = 6 mM; K_{ni2} = 6 mM; K_{ki1} = 20 mM; K_{ki2} = 30 mM; K_{ATP} = 0.08 mM; K_{ADP} = 2 mM; K_{pi} = 5 mM; K_{inact} = 1.9 or 0.24 s^{-1}.

Parameter settings for Fig. 7

Na exchange to the pipette tip was omitted, and the following parameter changes were employed: E_m = −80 mV; K_{inact} = 2 s^{-1}; K_{rec} = 40 s^{-1}; K_{pump} = 1 nA in absence of inactivation; K_{pump} = 4 nA with inactivation.

Na/H exchange simulations

For the simulations in Fig. 9, Na/H exchange was assumed to take place by a simple consecutive mechanism with the same extracellular and cytoplasmic Na and H dissociation constants (K_h and K_n), with the same Na and H translocation rates in both directions, and with Na and H binding occurring competitively. The fractions of bindings sites occupied by H and Na (F_{no}, F_{ni}, F_{ho}, and F_{hi}) were first calculated:

$D_o = 1 + N_o/K_n + H_o/K_h$, (28)

$F_{no} = N_o/K_n/D_o$, (29)

$F_{ho} = H_o/K_h/D_o$, (30)

$D_i = 1 + N_i/K_n + H_i/K_h$, (31)

$F_{ni} = N_i/K_n/D_i$, (32)

$F_{hi} = H_i/Kh/D_i$. (33)

Then, the fractions of transporters open to the outside (E_2) and inside (E_1) were calculated, as well as transport rate that is independent of inactivation, as a fractional maximal translocation rate (F_{max}):

$E_2 = (F_{ni} + F_{hi})/(F_{ni} + F_{hi} + F_{no} + F_{ho})$, (34)

$E_1 = 1\text{-}E_2$, (35)

$F_{max} = (E_2 \times F_{no}\text{-}E_1 \times F_{ni})$. (36)

The simulation assumes that Na must bind at the inactivated transport sites before cytoplasmic protons can bind to regulatory sites on the cytoplasmic side. It also includes the notion that both extracellular and cytoplasmic Na can be effective, assuming that inactive exchangers can be open to either the outside or inside. With a Na dissociation constant of 10 mM and a proton dissociation constant of 0.2 µM, the fraction of exchangers with 3 protons bound to regulatory sites (F_{3H}) is

$F_{3H} = (N_o+N_i)/10 \times H_i^3/0.2^3/(1 + (N_o+N_i)/10 \times (1 + H_i^3/0.2^3))$. (37)

Assuming that extracellular H binding to transport sites promotes inactivation (i.e., the opening of regulatory sites to the cytoplasmic side) at a rate that is twice faster than the maximal recovery from inactivation, the fraction of active exchangers (F_{act}) becomes

$$F_{act} = F_{3H/}(F_{3H} + F_{ho} \times 2). \quad (38)$$

And the fractional transport rate (R_{nhe}) is

$$R_{nhe} = F_{act} \times F_{max}. \quad (39)$$

Parameter settings for Fig. 9

N_i = 4 mM; $H_o = 0.1 \times 10^{-3}$ mM; $H_i = 0.12 \times 10^{-3}$ mM; K_n = 10 mM; $K_h = 0.1 \times 10^{-3}$ mM.