Biophysical Journal, Volume 97

Supporting Material

Differential Regulation of Action Potentials by Inactivating and Noninactivating BK Channels in Rat Adrenal Chromaffin Cells

Liang Sun, Yu Xiong, Xuhui Zeng, Ying Wu, Na Pan, Christopher Lingle, Anlian Qu, and Jiu Ping Ding

Supporting Material

Differential Regulation of Action Potentials by Inactivating and Noninactivating BK Channels in Rat Adrenal Chromaffin Cells

FIGURE S1 Definition and simulations of voltage-dependent Na^+ current in RCC. Chromaffin cell Na^+ channels are thought to be encoded by the $Na_V1.7$ and $\beta1/\beta3$ genes (1). For the $Na_V1.7+\beta1/\beta3$ channel, the half maximal-activation voltage (V_{50}) and the slope (κ) are -12.4 and 7.0 mV (3-4 days), -14.7 and 5.1 mV (11-15 days), respectively (2); the half availability voltage (V_{50}) and the slope (κ) are -49.3 and 9.8 mV (3-4 days), -42.8 and 8.6 mV (11-15 days) (2), respectively. The inactivation time constant (τ_I) is 1.5 ms at -10 mV (1). $Na_V1.7+\beta1/\beta3$ currents exhibit a double exponential time course of recovery from inactivation. At -100 mV, the ratio of fast mode to slow mode is about 4:1 (1). (*A*) The scheme defines the gating model and kinetic constants used for modeling Na^+ current. Parameter values for simulations are given in the associated table below. *(B1)* Traces show voltage-dependent Na^+ currents simulated based on the Scheme in A with parameters given in Supplementary Table 1. Simulations were based on the indicated voltage protocol (steps from -100 to +60 mV in 10 mV increments with a holding potential of -120; only every 4^{th} step is plotted) and run with the CeL software package. *(B2)*, The current-voltage curve for normalized Na^+ current amplitude is plotted as a function of command voltage. *(C1)* Traces show simulated Na^+ current utilizing a steady-state inactivation protocol in which currents at +10 mV are activated following 100 ms steps to potentials between -120 and 0 mV. *(C2)* The normalized fractional activation (○) and steady-state inactivation (●) curves for simulated Na^+ currents are plotted as a function of membrane potential. Solid lines are fits to the Boltzmann Eq. (1). The fitted values of V_{50} and slope factor for activation and steady-state inactivation are -17.5/5.1 mV and -39.8/-3.4 mV, respectively. *(D1)* Traces show simulated Na^+ current behavior with a paired pulse recovery protocol. *(D2)* The time course of fractional recovery of simulated Na^+ current is plotted as a function of three recovery potentials, -120, -50 and -40 mV. Solid lines were fitted to the Eg. (2). Recovery time constants (τ_r) are 2.4, 29.7 and 83.9 ms at –120, -50 and -40 mV, respectively.

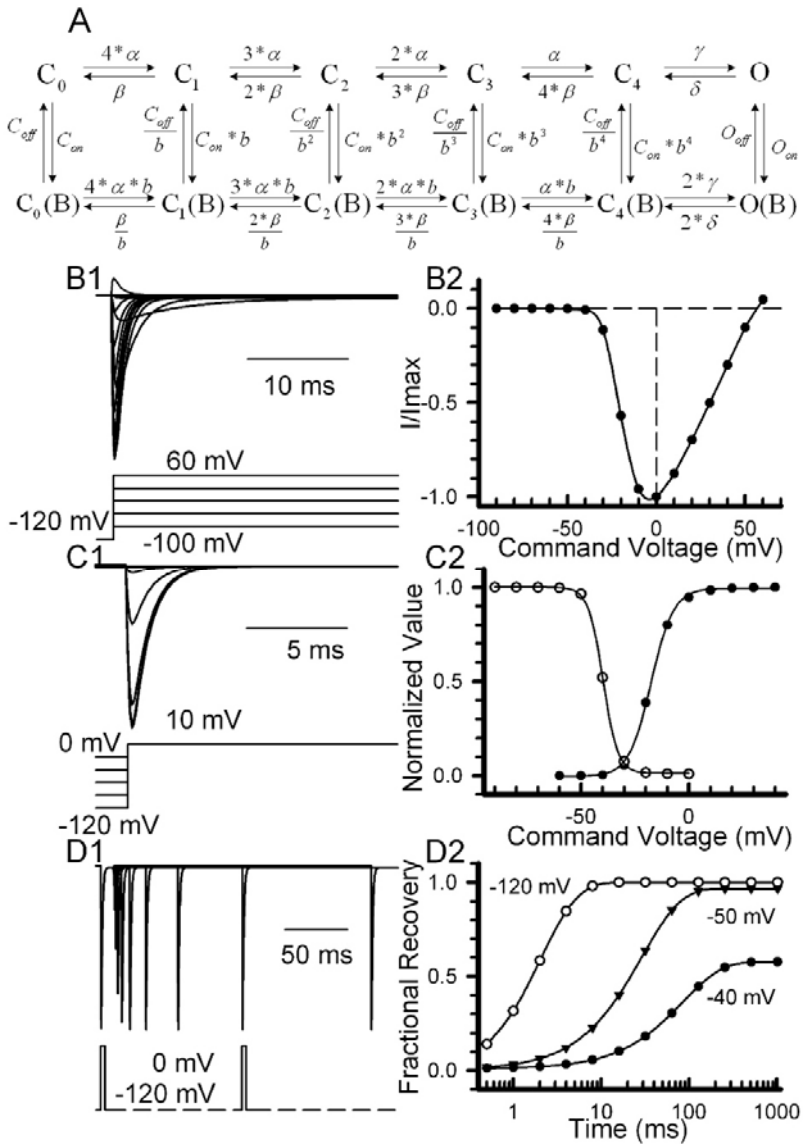


Table S1. Parameters used for Na^+ current simulations

$\alpha = A*\exp(z_1FV/RT)$	s^{-1}	$\beta = B*\exp(-z_2FV/RT)$	s^{-1}
A	288656	B	22145
z_1	2.154	z_2	0.539
Γ	7500	Δ	2000
C_{on}	0.5	C_{off}	500
O_{on}	900	O_{off}	6
		b	4.436203

FIGURE S2: Properties of simulated voltage-dependent K^+ current. (*A*) The scheme defines the gating model for Kv current behavior, with the definition of kinetic constants given on the bottom. *(B)* Simulated traces of Kv currents were obtained by voltage steps ranging from -80 to +120 mV with 20-mV increments from a holding potential of -100 mV. (*C*) The fractional activation (●) of simulated Kv currents is plotted as a function of membrane potential. Solid line is the best fit of the Boltzmann Eq. (1). The V_{50} and slope of activation are -0.8 and 11.1 mV, respectively. *(D)* The time constant of activation (τ_a) of Kv currents is plotted as a function of activation voltage.

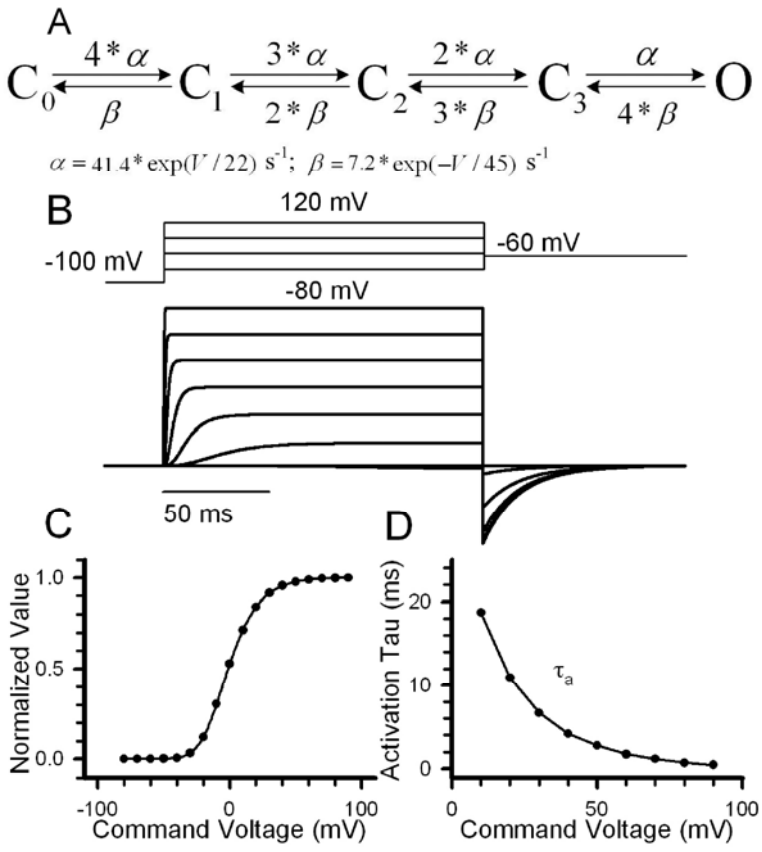


FIGURE S3 Properties of simulated voltage-activated Ca^{2+} current. (*A*) The scheme defines the gating model and kinetic constants used for simulation of HVA Ca^{2+} current. Parameters used for rate constants are defined in the table below. (*B*) Traces show high voltage-activated Ca^{2+} currents simulated based on the Scheme in A with parameters defined in Supplementary Table 2. Voltage steps ranged from -80 to +60 mV in 10-mV increments from a holding potential of -90 mV. (*C*) The normalized amplitudes of Ca^{2+} currents are plotted as a function of membrane potential. (*D*) The fractional activation (●) of Ca^{2+} current conductance is plotted as a function of membrane potential. Solid line is the best fit of the Boltzmann Eq. (1), with V_{50} = 2.3 mV (k= 6.0 mV). (*E*) The time constant (τ_a) of activation of Ca^{2+} current is plotted as a function of membrane potential.

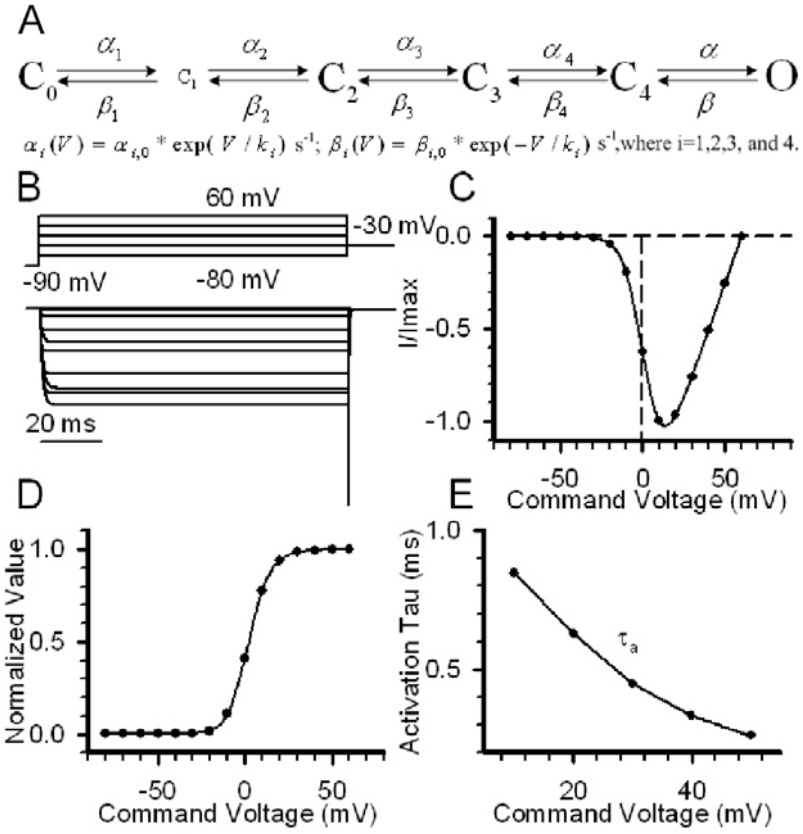


Table S2. Parameters Used for HVA Ca^{2+} current simulations

$\alpha_{i,0}$ (s^{-1})	$\beta_{i,0}$ (s^{-1})	k_i (mV)
$\alpha_{1,0}$=3989	$\beta_{1,0}$=5625	k_1 =68.75
$\alpha_{2,0}$=4617	$\beta_{2,0}$=7524	k_2 =39.53
$\alpha_{3,0}$=4892	$\beta_{3,0}$=75214	k_3 =281.62
$\alpha_{4,0}$=589305	$\beta_{4,0}$=907508	k_4 =18.46
α=615010	β=7680	

FIGURE S4 Simulation of noninactivating BK_s current. *(A)* Scheme shows kinetic model and definitions of rate constants used to define BK_s current behavior. (*B*) Traces on the left show BK_s currents simulated using the Scheme in A, with parameters given in Supplementary Table 3, in accordance with the indicated voltage protocol (top). (*C)* Simulated currents show the deactivation behavior of BK_s current. *(D)* The fractional values of activation of BK_s conductance are plotted as a function of membrane potential. The half maximal-activation voltages (V_{50}) are 157, 114, 65, 33 and 0 mV for ~2 nM (●), 1 μM (○), 4 μM (▼), 10 μM (△) and 60 μM (▬) Ca^{2+}, respectively. (*E)* The relationship between V_{50} vs $[Ca^{2+}]_i$ is displayed. (*F)* The activation (τ_a) and deactivation (τ_d) time constants for the BK_s current simulations are plotted as a function of voltage time constant-voltage for ~2 nM (●), 1 μM (○), 4 μM (▼), 10 μM (△) and 60 μM (▬).

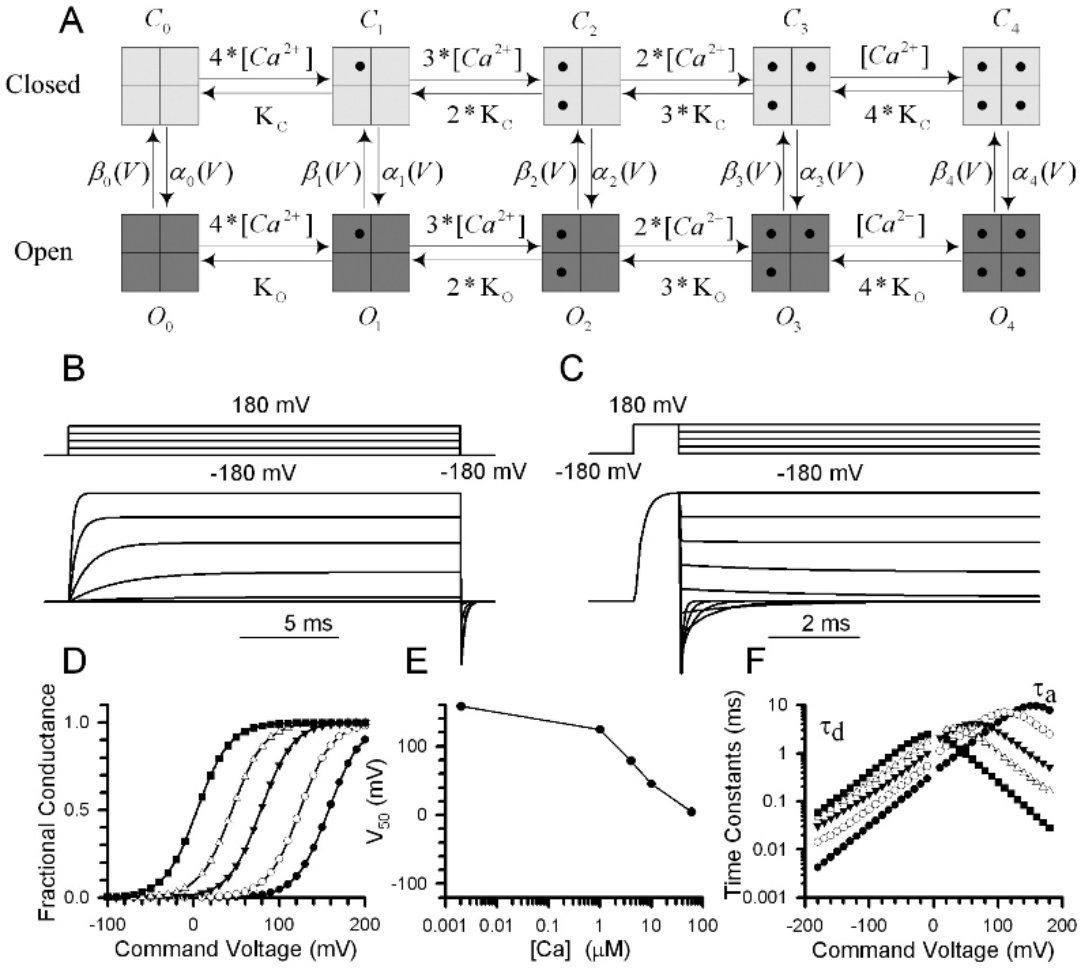
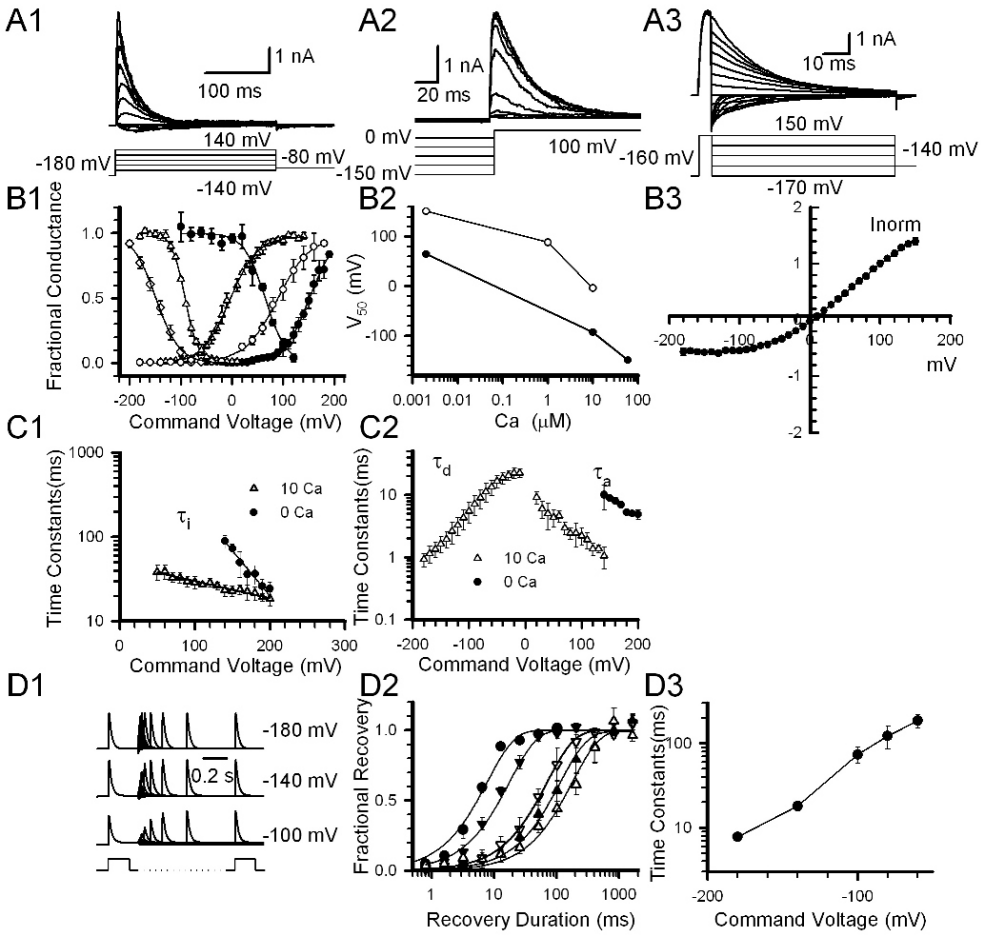


Table S3. Parameters Used for BK_s current simulations

$\alpha_n = A_n * \exp(z_{CO}FV / RT)$ s-1		$\beta_n = B_n * \exp(-z_{OC}FV / RT)$ s-1	
A_0	0.659	B_0	2651.7
A_1	3.955	B_1	1767.8
A_2	25.05	B_2	1244.0
A_3	129.2	B_3	713.0
A_4	261.1	B_4	160.0
z_{CO}	0.718	z_{OC}	0.646
K_C	13.5	K_O	1.5
Ca^{2+} on-rates per site		10^9 $M^{-1}s^{-1}$	
Ca^{2+} off-rates from C_n per binding site		$10^9 K_C$ (13,500 s^{-1})	
Ca^{2+} off-rates from O_n per binding site		$10^9 K_O$ (1,500 s^{-1})	

T=300 K, F=9.64853*10^4 C*mol^{-1}, R=8.31451 J*mol^{-1}*K^{-1}

FIGURE S5. Characteristics of BK_i (mSlo1+hβ2) current. *(A)* Families of current traces show activation (A1), steady-state inactivation (A2) and deactivation (A3) for BK_i channels (mSlo1+hβ2) obtained from inside-out patches with symmetrical 160 mM K^+ solutions and 10 μM internal Ca^{2+}. Currents were elicited by the voltage protocols shown below each set of traces. *(B1)* Fractional values of activation in ~2 nM (●), 1 μM (○) and 10 μM (△) Ca^{2+} and steady-state inactivation in ~2 nM (●), 10 μM (△) and 60 μM (◇) Ca^{2+} are plotted as a function of membrane potential. Solid lines are fits to the Boltzmann Eq. (1). (*B2*) The V_{50} of activation (○) and steady-state inactivation (●) are plotted as a function of $[Ca^{2+}]_i$. The V_{50}'s for activation are 151.3 mV (n=5), 88.4 mV (n=5) and -4.4 mV (n=4) in 2 nM, 1 μM and 10 μM Ca^{2+}, respectively. The V_{50}'s for steady-state inactivation are 64.5 mV (n=5), -92.6 mV (n=4) and –149.2 mV (n=4) in 2 nM, 10 μM and 60 μM Ca^{2+}, respectively. (*B3*) The normalized instantaneous tail currents of BK_i channels are plotted as a function of voltage. *(C1)* Inactivation time constant (τ_i) is plotted as a function of voltage for 2 nM (●) and 10 μM (△) Ca^{2+}. (*C2*) Deactivation time constants (τ_d) are plotted as a function of voltage for 2 nM (●) and 10 μM (△) Ca^{2+} and the activation time constant (τ_a) is plotted as a function of voltage for 2 nM (●), 1 μM (○), 4 μM (▼) and 10 μM (△). *(D1)* Traces show recovery of BK_i current amplitude elicited by a paired pulse protocol (activation steps to 100 mV) separated by steps of different duration to -180, -140 and -100 mV. The voltage protocol is shown at the bottom. (*D2*) The time course of recovery from inactivation is plotted for BK_i current for recovery voltages of -180, -140, -100, -80 and -60 mV, in 10 μM Ca^{2+} (n=4). Solid lines were the best fits of Eq. (2). Recovery time constants (τ_r) are 7.8, 18.1, 73.5, 122.3 and 185.3 ms at -180, -140, -100, -80 and -60 mV, respectively. (*D3*) The dependence of τ_r on recovery voltage is displayed for 10 μM Ca^{2+}.



Appendix

The first-order ordinary differential equation for determination of membrane potential. For each component, *i*, of conductance, we assumed that the flow of ions behaves ohmically (3):

$$I_i = G_i(V,[Ca^{2+}]) \times (V - E_i)$$

where I_i is the ionic current in pA of component *i*, $G_i(V, [Ca^{2+}])$ is the dependence of conductance on voltage and/or calcium, measured in nS, E_i is the reversal potential in mV and V is the membrane voltage, in mV.
Similarly, the leak current of cell membrane can be written as follows:

$$I_L = G_L \times (V - E_L)$$

where G_L is a constant conductance in nS, and E_L is the reversal potential in mV.

Using Kirchhoff's laws, one can write the differential equation for the total current flowing across the membrane, which is the sum of the capacitive currents of insulating cell membrane $I_C = C_m \times dV/dt$, the ionic current $I_{ion} = \sum I_{Na} + I_K + I_{Ca} + \ldots$, the leak current I_L, and the injection current I_{Inj}. Consequently, the evolution of the membrane voltage in time is described by the first-order ordinary differential equation:

$$C_m \times dV/dt = I_{Inj} + I_L + \sum G_i(V,[Ca^{2+}]) \times (V - E_i) \quad \text{(A1)}$$

where C_m is the capacity of the cell membrane, measured in pF.
Unlike the Hodgkin-Huxley formalism, the whole-cell conductance of each ionic current is described by the following:

$$G_i(V,[Ca^{2+}]) = n \times \hat{g}_i \times P_o(V,[Ca^{2+}]) \quad \text{(A2)}$$

where $P_o(V, [Ca^{2+}])$ is the open probability of channels, n is the number of ion channels, and $\hat{g}_i$ is the single-channel conductance. The instantaneous P_o of Na^+, Ca^{2+}, Kv and BK currents was calculated based on their kinetic models.

1. Morgan, K., E. B. Stevens, B. Shah, P. J. Cox, A. K. Dixon, K. Lee, R. D. Pinnock, J. Hughes, P. J. Richardson, K. Mizuguchi, and A. P. Jackson. 2000. β3: an additional auxiliary subunit of the voltage-sensitive sodium channel that modulates channel gating with distinct kinetics. *Proc. Natl. Acad. Sci. USA.* 97:2308-2313.
2. Islas-Suarez, L., M. Gomez-Chavarin, R. Drucker-Colin, and A. Hernandez-Cruz. 1994. Properties of the sodium current in rat chromaffin cells exposed to nerve growth factor in vitro. *J Neurophysiol.* 72:1938-1948.
3. Hille, B. 2001. Ionic Channels of Excitable Membranes. Sinauer Associates Inc., Sunderland Massachusetts.