Hu et al., http://www.jgp.org/cgi/content/full/jgp.201311013/DC1

Derivation of gating of HCN2 channel by voltage, cAMP, and TRIP8b

According to the 12-state model (Fig. 6), the open probability of HCN2 channels as a function of voltage (V), cAMP concentration (A), and TRIP8b concentration (T) is given by

$$P_V(A,T) = \frac{\sum O}{\sum O + \sum C_R + \sum C_A}$$

$$= \frac{1}{1 + L\cdot\left(1+K_V\right)\cdot\dfrac{1+\dfrac{A}{K_C^A}+\dfrac{T}{K_C^T}+\dfrac{A\cdot T}{K_C^T\cdot K_{TC}^A}}{1+\dfrac{A}{K_O^A}+\dfrac{T}{K_O^T}+\dfrac{A\cdot T}{K_O^T\cdot K_{TO}^A}}}. \quad (1)$$

All the terms are defined in Fig. 6, with K_V, the equilibrium constant for channel activation, given by

$$K_V = Q_0\cdot e^{V/s},$$

where Q_0 is the equilibrium constant for channel activation at 0 mV and s is the slope factor of the Boltzmann activation curve (Zhou and Siegelbaum, 2007).

Solving $\Delta V_{1/2}$ as a function of cAMP and TRIP8b concentrations

At the voltage where the channel is half activated ($V_{1/2}$), we write

$$P_{V_{1/2}}(A,T) = \frac{1}{2}\cdot P_\infty(A,T) \quad (2)$$

$$= \frac{1}{2}\cdot\frac{1}{1 + L\cdot\dfrac{1+\dfrac{A}{K_C^A}+\dfrac{T}{K_C^T}+\dfrac{A\cdot T}{K_C^T\cdot K_{TC}^A}}{1+\dfrac{A}{K_O^A}+\dfrac{T}{K_O^T}+\dfrac{A\cdot T}{K_O^T\cdot K_{TO}^A}}}, \quad (3)$$

where $P_\infty(A,T)$ is the maximal open probability of the channel at extreme negative voltages where activation gating is complete.

Combining Eqs. 1 and 3 yields

$$1 + L\cdot\left(1+K_{V_{1/2}}\right)\cdot\frac{1+\dfrac{A}{K_C^A}+\dfrac{T}{K_C^T}+\dfrac{A\cdot T}{K_C^T\cdot K_{TC}^A}}{1+\dfrac{A}{K_O^A}+\dfrac{T}{K_O^T}+\dfrac{A\cdot T}{K_O^T\cdot K_{TO}^A}}$$

$$= 2\cdot\left(1 + L\cdot\frac{1+\dfrac{A}{K_C^A}+\dfrac{T}{K_C^T}+\dfrac{A\cdot T}{K_C^T\cdot K_{TC}^A}}{1+\dfrac{A}{K_O^A}+\dfrac{T}{K_O^T}+\dfrac{A\cdot T}{K_O^T\cdot K_{TO}^A}}\right).$$

We can thus derive $K_{V_{1/2}}$:

$$K_{V_{1/2}} = 1 + \frac{1}{L}\cdot\frac{1+\dfrac{A}{K_O^A}+\dfrac{T}{K_O^T}+\dfrac{A\cdot T}{K_O^T\cdot K_{TO}^A}}{1+\dfrac{A}{K_C^A}+\dfrac{T}{K_C^T}+\dfrac{A\cdot T}{K_C^T\cdot K_{TC}^A}} = Q_0\cdot e^{\frac{V_{1/2}}{s}}.$$

$V_{1/2}$ can then be solved as a function of cAMP concentration (A) and TRIP8b concentration (T) from the following:

$$V_{1/2}(A,T) =$$

$$s\cdot\ln\left(1 + \frac{1}{L}\cdot\frac{1+\dfrac{A}{K_O^A}+\dfrac{T}{K_O^T}+\dfrac{A\cdot T}{K_O^T\cdot K_{TO}^A}}{1+\dfrac{A}{K_C^A}+\dfrac{T}{K_C^T}+\dfrac{A\cdot T}{K_C^T\cdot K_{TC}^A}}\right) - s\cdot\ln Q_0.$$

When cAMP is absent, the expression becomes

$$V_{1/2}(0,T) = s\cdot\ln\left(1 + \frac{1}{L}\cdot\frac{1+\dfrac{T}{K_O^T}}{1+\dfrac{T}{K_C^T}}\right) - s\cdot\ln Q_0.$$

Thus, we can now solve for the change in $V_{1/2}$ in the presence of cAMP as

$$\Delta V_{1/2}(A,T) = V_{1/2}(A,T) - V_{1/2}(0,T)$$

$$= s\cdot\left[\ln\left(1 + \frac{1}{L}\cdot\frac{1+\dfrac{A}{K_O^A}+\dfrac{T}{K_O^T}+\dfrac{A\cdot T}{K_O^T\cdot K_{TO}^A}}{1+\dfrac{A}{K_C^A}+\dfrac{T}{K_C^T}+\dfrac{A\cdot T}{K_C^T\cdot K_{TC}^A}}\right) - \ln\left(1 + \frac{1}{L}\cdot\frac{1+\dfrac{T}{K_O^T}}{1+\dfrac{T}{K_C^T}}\right)\right]. \quad (4)$$

We used Eq. 4 in the model fitting in Fig. 7 to solve for $\Delta V_{1/2}$ as a function of cAMP and TRIP8b concentrations.

Solving reduction of the maximal tail current as a function of TRIP8b concentration

As shown in Eqs. 2 and 3, at extreme negative voltages, the maximal open probability of the channel as a function of cAMP and TRIP8b concentrations is given by

$$P_\infty(A,T) = \frac{1}{1 + L \cdot \frac{1 + \frac{A}{K_C^A} + \frac{T}{K_C^T} + \frac{A \cdot T}{K_C^T \cdot K_{TC}^A}}{1 + \frac{A}{K_O^A} + \frac{T}{K_O^T} + \frac{A \cdot T}{K_O^T \cdot K_{TO}^A}}}.$$

In the absence of cAMP, the expression becomes

$$P_\infty(0,T) = \frac{1}{1 + L \cdot \frac{1 + \frac{T}{K_C^T}}{1 + \frac{T}{K_O^T}}}. \quad (5)$$

In the absence of both cAMP and TRIP8b, we obtain

$$P_\infty(0,0) = \frac{1}{1+L}. \quad (6)$$

Combining Eqs. 5 and 6 yields

$$\frac{P_\infty(0,T)}{P_\infty(0,0)} = \frac{1+L}{1 + L \cdot \frac{1 + \frac{T}{K_C^T}}{1 + \frac{T}{K_O^T}}}.$$

Thus, the current reduction caused by the $TRIP8b_{core}$ polypeptide can be described from the following relationship:

$$1 - \frac{P_\infty(0,T)}{P_\infty(0,0)} = 1 - \frac{1+L}{1 + L \cdot \frac{1 + \frac{T}{K_C^T}}{1 + \frac{T}{K_O^T}}}.$$

Procedures of model fitting

We recorded HCN2 channel currents at various voltages in the presence of 0–1,000 µM cAMP and 0–12 µM $TRIP8b_{core}$ peptides and thus obtained the relationship among $\Delta V_{1/2}$, [cAMP], and [$TRIP8b_{core}$] (Fig. 7). We adjusted the parameters of the model to obtain the best fit to the measured relation between [cAMP] and $\Delta V_{1/2}$, in the presence of 0–12 µM $TRIP8b_{core}$ peptide. At 0 $TRIP8b_{core}$ peptide, the model returns to the front face (Fig. 6), which is the six-state cyclic model proposed in Zhou and Siegelbaum (2007). Thus, we adopted the dissociation constants for cAMP binding to the closed (K_C^A) and open states (K_O^A) of the channel, as well as the equilibrium constants for the voltage-dependent activation step (K_V) and voltage-independent opening step (L) directly from Zhou and Siegelbaum (2007), which provided a good fit to our data in the absence of TRIP8b. We then fit the data in the presence of TRIP8b by adjusting four free parameters, the dissociation constants for TRIP8b binding to the closed state (K_C^T), open state (K_O^T), cAMP-bound closed state (K_{AC}^T), and cAMP-bound open state (K_{AO}^T). Because of the cyclic nature of the model, the other equilibrium constants are derived from the above parameters.

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