Supplemental Material

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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

\[
P_v(A,T) = \frac{1}{1 + L \cdot (1 + K_v)} = \frac{1}{1 + \frac{A}{K^A} + \frac{T}{K^T} + \frac{A \cdot T}{K^A \cdot K^T}}
\]

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

\[
P_{V_{1/2}}(A,T) = \frac{1}{2} \cdot P_v(A,T)
\]

\[
= \frac{1}{\frac{1}{2} \cdot \frac{1}{1 + \frac{A}{K^A} + \frac{T}{K^T} + \frac{A \cdot T}{K^A \cdot K^T}}}
\]

\[
= \frac{1 + \frac{A}{K^A} + \frac{T}{K^T} + \frac{A \cdot T}{K^A \cdot K^T}}{1 + \frac{A}{K^A} + \frac{T}{K^T} + \frac{A \cdot T}{K^A \cdot K^T}}
\]

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

Combining Eqs. 1 and 3 yields

\[
1 + L \cdot (1 + K_{V_{1/2}}) \cdot \frac{1 + \frac{A}{K^A} + \frac{T}{K^T} + \frac{A \cdot T}{K^A \cdot K^T}}{1 + \frac{A}{K^A} + \frac{T}{K^T} + \frac{A \cdot T}{K^A \cdot K^T}} = 2 \cdot \frac{1 + \frac{A}{K^A} + \frac{T}{K^T} + \frac{A \cdot T}{K^A \cdot K^T}}{1 + \frac{A}{K^A} + \frac{T}{K^T} + \frac{A \cdot T}{K^A \cdot K^T}}
\]

We can thus derive \(K_{V_{1/2}}\):

\[
K_{V_{1/2}} = 1 + \frac{1}{L} \cdot \frac{1 + \frac{A}{K^A} + \frac{T}{K^T} + \frac{A \cdot T}{K^A \cdot K^T}}{1 + \frac{A}{K^A} + \frac{T}{K^T} + \frac{A \cdot T}{K^A \cdot K^T}} = Q_0 \cdot e^{V_{1/2}/s}
\]

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

\[
V_{1/2}(A,T) = s \cdot \ln \left(1 + \frac{1}{L} \cdot \frac{1 + \frac{A}{K^A} + \frac{T}{K^T} + \frac{A \cdot T}{K^A \cdot K^T}}{1 + \frac{A}{K^A} + \frac{T}{K^T} + \frac{A \cdot T}{K^A \cdot K^T}}\right) - s \cdot \ln Q_0.
\]

When cAMP is absent, the expression becomes

\[
V_{1/2}(0,T) = s \cdot \ln \left(1 + \frac{1}{L} \cdot \frac{1 + \frac{T}{K^T}}{1 + \frac{T}{K^T}}\right) - s \cdot \ln Q_0.
\]

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

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

\[
= s \cdot \ln \left(1 + \frac{1}{L} \cdot \frac{1 + \frac{A}{K^A} + \frac{T}{K^T} + \frac{A \cdot T}{K^A \cdot K^T}}{1 + \frac{A}{K^A} + \frac{T}{K^T} + \frac{A \cdot T}{K^A \cdot K^T}}\right) - s \cdot \ln \left(1 + \frac{1}{L} \cdot \frac{1 + \frac{T}{K^T}}{1 + \frac{T}{K^T}}\right)
\]
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

$$P_{\infty}(A,T) = \frac{1}{1 + \frac{A \cdot T}{K_C} + \frac{A \cdot T}{K_O} + \frac{A \cdot T}{K_N} \cdot \frac{A \cdot T}{K_D}}.$$  

In the absence of cAMP, the expression becomes

$$P_{\infty}(0,T) = \frac{1}{1 + \frac{T}{K_C} + \frac{T}{K_O} + \frac{T}{K_D}}.$$  \hspace{1cm} (5)

In the absence of both cAMP and TRIP8b, we obtain

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

Combining Eqs. 5 and 6 yields

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

Thus, the current reduction caused by the TRIP8b core polypeptide can be described from the following relationship:

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

**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 ($K_C^{AC}$), and cAMP-bound open state ($K_O^{AO}$). Because of the cyclic nature of the model, the other equilibrium constants are derived from the above parameters.

**Reference**

Zhou, L., and S.A. Siegelbaum. 2007. Gating of HCN channels by cyclic nucleotides: residue contacts that underlie ligand binding, selectivity, and efficacy. *Structure*. 15:655–670. http://dx.doi.org/10.1016/j.str.2007.04.012