Supplemental Material

Babich et al., http://www.jgp.org/cgi/doi/10.1085/jgp.200709733

A

\[
\begin{align*}
\text{[Ca]/K}_{D,\text{Ca}} & \quad \text{K}_{\text{Ca}} \\
\text{Ca} & \quad \text{Ca} \\
\text{[Na]/K}_{D,\text{Na}} & \quad \text{K}_{\text{Na}} \\
\text{Na} & \quad \text{Na} \\
\text{ONa} & \quad \text{CaNa} \\
\text{NaNa} & \quad \text{NaCa}
\end{align*}
\]

B

**Ca\(^{2+}\) block of Na\(^{+}\) currents**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Occupancy</th>
<th>[Ca]</th>
<th>[Na]</th>
<th>K(_{D,\text{Ca}})</th>
<th>K(_{D,\text{Na}})</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>0.01</td>
<td>0.001</td>
<td>10</td>
<td>1000</td>
<td>0.01</td>
</tr>
<tr>
<td>EEQE</td>
<td>0.001</td>
<td>0.0001</td>
<td>10</td>
<td>1000</td>
<td>0.01</td>
</tr>
</tbody>
</table>

C

**Tonic block of Ca\(^{2+}\) currents by Gd\(^{3+}\)**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Occupancy</th>
<th>[Gd]</th>
<th>[Ca]</th>
<th>K(_{D,\text{Gd}})</th>
<th>K(_{D,\text{Ca}})</th>
</tr>
</thead>
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<td>0.1</td>
<td>1000</td>
</tr>
<tr>
<td>EEQE</td>
<td>0.5</td>
<td>0.01</td>
<td>0.01</td>
<td>0.1</td>
<td>1000</td>
</tr>
</tbody>
</table>

D

**Use-dependent block by Gd\(^{3+}\)**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Occupancy</th>
<th>[Gd]</th>
<th>[Ca]</th>
<th>K(_{D,\text{Gd}})</th>
<th>K(_{D,\text{Ca}})</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>0.5</td>
<td>10</td>
<td>10</td>
<td>0.1</td>
<td>1000</td>
</tr>
<tr>
<td>EEQE</td>
<td>0.5</td>
<td>0.01</td>
<td>0.01</td>
<td>0.1</td>
<td>1000</td>
</tr>
</tbody>
</table>
Figure S1. Steady-state properties of a two-site single-file mechanism of competition between two kinds of ions. (A) The kinetic scheme. Ions can bind only to the unoccupied entry site (to states OO, OCa, and ONa). The equilibrium constants for all binding steps are \([\text{Ca}] / K_{D,\text{Ca}}\) and \([\text{Na}] / K_{D,\text{Na}}\), depending on the type of ion. Ions can occupy the internal (“high affinity”) site by stepping from the entry site. The equilibrium constants for the transfer steps are \(K_{\text{Ca}}\) and \(K_{\text{Na}}\).

The occupancies of the states are calculated by:

\[
\begin{align*}
\text{OO} &= 1 / \Pi, \\
\text{OCa} &= \frac{\kappa_{\text{Ca}}}{\Pi}, \\
\text{OCa} &= \frac{\kappa_{\text{Ca}} K_{\text{Ca}}}{\Pi}, \\
\text{CaO} &= \frac{\kappa_{\text{Ca}}}{\Pi}, \\
\text{ONa} &= \frac{\kappa_{\text{Na}}}{\Pi}, \\
\text{NaO} &= \frac{\kappa_{\text{Na}}}{\Pi}, \\
\text{NaCa} &= \frac{\kappa_{\text{Ca}} \kappa_{\text{Na}}}{\Pi}, \\
\text{NaN} &= \frac{\kappa_{\text{Na}}^2}{\Pi}, \\
\Sigma \text{Ca} &= \text{CaO} + \text{OCa} + \text{CaCa} + \text{CaNa} + \text{NaCa},
\end{align*}
\]

where \(\kappa_{\text{Na}} = [\text{Na}] / K_{D,\text{Na}}\) and \(\kappa_{\text{Ca}} = [\text{Ca}] / K_{D,\text{Ca}}\). \(\Pi = (1 + \kappa_{\text{Ca}})(1 + \kappa_{\text{Ca}} K_{\text{Ca}} + \kappa_{\text{Na}})\).

(B) Simulation of block of Na\(^{+}\) currents by Ca\(^{2+}\). Because diffusion of sub-\(\mu\)M of Ca\(^{2+}\) is slow in comparison with activation kinetics, the major part of Na\(^{+}\) current block is determined by Ca\(^{2+}\) binding to closed channels. According to the findings in Figs. 7 and 8, the affinity of the entry site to Ca\(^{2+}\)/Ba\(^{2+}\) is about 1 mM in closed channels. Thus, \(\kappa_{\text{Ca}} = [\text{Ca}] / K_{D,\text{Ca}} = 1 \mu M / 1 mM = 0.001\). In order for the internal site to be nearly half-occupied at 1 \(\mu\)M Ca\(^{2+}\), \(K_{\text{Ca}} = 1,000\). The affinity of the entry site for Na\(^{+}\) is supposed to be \(\sim 10\) mM (\(\kappa_{\text{Na}} = 10\)) and the selectivity site is supposed not to bind Na\(^{+}\) (\(\kappa_{\text{Na}} = 0.01\)). In this condition, about half of channels are occupied by Ca\(^{2+}\) (left). If the EEQE mutant reduces the affinity of the entry site for Ca\(^{2+}\), most of channels are unoccupied by Ca\(^{2+}\) because the entry into the selectivity site is predominantly occupied by Na\(^{+}\) (right). (C) To simulate tonic block of Ca\(^{2+}\) currents by Gd\(^{3+}\), the same scheme is used. Here, Ca\(^{2+}\) plays the role of Na\(^{+}\) in B. Although \(K_{D,\text{Ca}}\) and \(K_{\text{Ca}}\) are the same as before, \([\text{Ca}] = 10\) mM, thus: \(\kappa_{\text{Ca}} = [\text{Ca}] / K_{D,\text{Ca}} = 10\). Parameters for Gd\(^{3+}\), \(\kappa_{\text{Gd}} = [\text{Gd}] / K_{D,Gd} = \frac{0.1 \mu M}{0.01 \mu M} = 10\), \(K_{\text{Ca}} = 0.1\), correspond to that it binds predominantly to the entry site. (D) Use-dependent block of Ca\(^{2+}\) currents by Gd\(^{3+}\) is proposed to occur because the affinity of the entry site to Ca\(^{2+}\) decreases as a result of activation: \(\kappa_{\text{Ca}} = 1\) (10-fold less than in C). This leads to an increase of the occupancy of the entry site by Gd\(^{3+}\). The mutant that affects the entry site reduces the potency of Gd\(^{3+}\) for both tonic and use-dependent effects.