SUPPLEMENTARY MATERIAL

Pflügers Archiv European Journal of Physiology

Methods for quantification of pore – voltage-sensor interaction in CaV1.2

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**Reduction of the 4-state circular model**

In accordance with a concept of “the rate limiting stage” the fastest transitions might be analysed as being in quasy equilibrium condition. In our model we assume that these fast transitions are $R \leftrightarrow A$ and $O \leftrightarrow D$:

\[
R \cdot x(V) \equiv A \cdot y(V) \\
D \cdot u(V) \equiv O \cdot w(V)
\]

\[\text{1/}\]

\[
\Psi = A + R \\
\Omega = O + D
\]

\[\text{2/}\]

From equations /1/ and /2/:

\[
R = \Psi \frac{1}{1 + \frac{x}{y}}, \quad A = \Psi \frac{1}{1 + \frac{y}{x}}, \quad O = \Omega \frac{1}{1 + \frac{w}{u}}, \quad D = \Omega \frac{1}{1 + \frac{u}{w}}
\]

Transition from closed ($\Psi = A + R$) to open ($\Omega = O + D$) states:

\[
\frac{d\Omega}{dt} = \alpha \cdot A - \beta \cdot O + \gamma \cdot R - \delta \cdot D = \]

\[
= \alpha \cdot \Psi \frac{1}{1 + \frac{y}{x}} - \beta \cdot \Omega \frac{1}{1 + \frac{w}{u}} + \gamma \cdot \Psi \frac{1}{1 + \frac{x}{y}} - \delta \cdot \Omega \frac{1}{1 + \frac{u}{w}}
\]

Condition $\Omega + \Psi = 1$ yields:
\[
\frac{d\Omega}{dt} = \left[ \frac{\alpha + \gamma}{1 + \frac{y}{x}} + \frac{\alpha + \beta + \gamma + \delta}{1 + \frac{x}{u} + \frac{x}{y} + \frac{u}{w}} \right] \Omega \quad /3/
\]

This equation predicts a mono-exponential time course with a time constant:

\[
\tau_m = \left( \frac{\alpha + \beta + \gamma + \delta}{1 + \frac{y}{x} + \frac{x}{u} + \frac{x}{y} + \frac{u}{w}} \right)^{-1} \quad /4/
\]

where \( y = \frac{y_0}{x_0} \cdot \exp(-\frac{V}{k_S}) \), \( w = \frac{y_0}{x_0} \cdot \frac{\beta}{\alpha} \cdot \frac{\gamma}{\delta} \cdot \exp(-\frac{V}{k_S}) \) (see [4])

The steady-state activation curve is described as in Beyl et al. [4]:

\[
m_\infty = \frac{1}{1 + \exp\left( \frac{V_m-V}{k_S} \right)} \quad /5/
\]

with midpoint: \( V_m = V_X - k_S \cdot \ln \left( 1 + \frac{\alpha}{\beta} \right) \approx V_X - k_S \cdot \ln \left( 1 + \frac{\alpha}{\beta} \right) \) \( /6/\)

and slope factor \( k_S = \frac{k_x \cdot k_y}{k_x + k_y} \) and \( V_X = k_S \cdot \ln \left( \frac{V_0}{x_0} \right) \) \( /7/\).

Formulæ /4/ - /7/ were used for estimation of the model parameters.

Asymptotic estimation of the model parameters

Asymptotic estimations of voltage dependence of \( \tau_m \) yields:

At strong depolarization \( (V \to +\infty) \):

\[
x/y \to +\infty \text{ and } u/w \to +\infty
\]

\[
y/x \to 0 \text{ and } w/u \to 0 \text{ that yields}
\]

\[
rate(depolarization) = \frac{1}{\tau_m(depolarization)} \to \alpha + \beta
\]
At strong hyperpolarization \((V \to -\infty)\):
\[
\frac{y}{x} \to +\infty \text{ and } \frac{w}{u} \to +\infty \\
\frac{x}{y} \to 0 \text{ and } \frac{u}{w} \to 0 \text{ that yields}
\]
\[
rate(hyperpolarization) = \frac{1}{\tau_m(hyperpolarization)} \to \gamma + \delta
\]

Figure 1 illustrates that all items in the sum \(rates\) reach their asymptotic values at physiological membrane voltages (exemplified for wild type and mutant G1193T).

At \(V = V_m\) the impacts of particular rates into the resulting rate are (formulae deduced under assumption that \(\gamma \ll \delta\)):
\[
rate_{\alpha,V} = \frac{\alpha}{1 + \frac{\alpha}{\beta}}, \quad rate_{\beta,V} = \beta, \quad rate_{\gamma,V} = \frac{\gamma(1 + \frac{\alpha}{\beta})}{2 + \frac{\alpha}{\beta}}, \quad rate_{\delta,V} = \gamma(1 + \frac{\alpha}{\beta})
\]

Major impact into resulting rate (sum of particular rates) is introduced by \(rate_{\beta,V}\). Thus, time constant at potentials near the middle of the activation curve \((V_m)\) is approximately equal to \(1/\beta\).
Illustration of the unique identification of the model parameters

**Figure 2. Graphical illustration of parameter identifiability**

Plot shows the level sets of the logarithm of the objective function $J(q)$ (see eg. /1/ in Results section) in the neighborhood of the identified parameter marked by the cross (center of the graph). Two out of the six model parameters are varied according to the axis labels, the remaining parameters are fixed by their values. The closed lines indicate pronounced curvatures in the parameter landscape and hence (at least local) uniqueness of identified parameters. Similar observations are made for other parameter variations.