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A novel biophysical model on calcium and voltage dual dependent gating of calcium-activated chloride channel

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HIGHLIGHTS

- A novel biophysical model for gating of CaCCs.
- Native CaCCs share universal Ca^{2+}- and voltage-dependent gating mechanism.
- Ca^{2+} facilitates opening of CaCCs by reducing the energy barrier of gating.
- TMEM16A shows different Ca^{2+} dependence with native CaCCs.

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ABSTRACT

Ca^{2+}-activated Cl^- channels (CaCCs) are anion-selective channels and involved in physiological processes such as electrolyte/fluid secretion, smooth muscle excitability, and olfactory perception which critically depend on the Ca^{2+} and voltage dual-dependent gating of channels. However, how the Ca^{2+} and voltage regulate the gating of CaCCs still unclear. In this work, the authors constructed a biophysical model to illustrate the dual-dependent gating of CaCCs. For validation, we applied our model on both native CaCCs and exogenous TMEM16A which is thought to be the molecular basis of CaCCs. Our data show that the native CaCCs may share universal gating mechanism. We confirmed the assumption that by binding with the channel, Ca^{2+} decreases the energy-barrier to open the channel, but not changes the voltage-sensitivity. For TMEM16A, our model indicates that the exogenous channels show different Ca^{2+} dependent gating mechanism from the native ones. These results advance the understanding of intracellular Ca^{2+} and membrane potential regulation in CaCCs, and shed new light on its function in aspect of physiology and pharmacology.

1. Introduction

Ca^{2+}-activated Cl^- channels (CaCCs) are anion-selective channels that are activated by increases in cytosolic free Ca^{2+} due to release from intracellular stores or influx through plasma membrane channels. CaCCs were first described in 1983 in *Xenopus* oocytes (Barish, 1983), and they have subsequently been identified in epithelial cells, vascular endothelial cells, neurons, and cardiac muscle cells. CaCCs are involved in diverse physiological processes such as electrolyte/fluid secretion, smooth muscle excitability, and olfactory perception (Kunzelmann et al., 2009).

One of the most common features of native CaCCs in various tissues and cell types is a characteristic voltage dependence that is modulated by intracellular free Ca^{2+} (Hartzell et al., 2005; Frings et al., 2000; Kuruma and Hartzell, 2000; Evans and Marty, 1986). At nanomolar Ca^{2+} concentrations, such CaCCs are activated and deactivated by positive and negative membrane potentials, respectively. Consequently, the steady state current–voltage relationship is strongly outwardly rectifying. At higher micromolar Ca^{2+} concentrations, on the other hand, the channels are fully activated at all membrane potentials and the steady state current–voltage relationship becomes linear (Galietta, 2009). It can be deduced that CaCCs can exist in any of the three states: close, partial open and full open state (Boese et al., 2004). When no evident current is shown, the channels are in the closed state. When the outwardly rectifying current is present, the channels are in partial open state. When the non-rectifying current is shown at all membrane potentials, the channels are in full open state. But unlike typical voltage-gated channels or ligand-gated channels, CaCCs exhibit both voltage dependence and ligand gating that are strongly coupled and apparently reciprocally related (Xiao et al., 2011).

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Opening (activation) and closing (deactivation and inactivation) of ion channels are the fundamental processes that underlie their physiological behavior. Consequently, understanding the mechanism of gating of ion channels is an important goal in comprehending the roles they play in cellular functions. Since 1983, research into CaCCs has, for a long time, relied heavily on carefully controlled electrophysiology experiments. However, the biophysical properties of these channels have not been studied sufficiently to address the question of activation mechanisms. Therefore, at minimum, exactly how these channels are controlled by Ca^{2+} and voltage is required. In 1996, Arreola et al. (1996) first developed a kinetic model (Appendix A) to account for the steady state current–voltage relationship of CaCCs in rat parotid acinar cells. In this model, channel activation involves two identical, independent, sequential Ca^{2+} binding steps before a final Ca^{2+}-independent transition to the conducting conformation, and the steady state open probability is Ca^{2+}-dependent and also includes two voltage-dependent equilibrium constants. The model in Arreola et al. (1996) is able to provide a reasonable simulation of activation kinetics of macroscopic Cl^- currents during the voltage clamp step at different Ca^{2+} concentrations and it has been used to describe the movement of Cl^- in different tissues and cell types (Maclaren et al., 2012; Tong et al., 2011; Palk et al., 2010). But the model in Arreola et al. (1996) could not reflect how Ca^{2+} influence the voltage dependence, and it cannot clarify what role Ca^{2+} and voltage play in the gating progress of CaCCs.

In order to illustrate the Ca^{2+} and voltage dual dependent gating mechanism, the authors proposed a theoretical model which reproduces all the major electrophysiological features of both native CaCCs and exogenous TMEM16A. We validated our model on two native CaCCs and two exogenous TMEM16A channel. Our data illustrate how the Ca^{2+} and voltage couple to regulate the gating of CaCCs. We demonstrate that the native and exogenous CaCCs may share different gating mechanism. The model may apply on reproducing the kinetic data of CaCCs or other Ca^{2+}-voltage dependent channels.

2. Model

Macroscopic currents recorded from whole cell or excised patches represent the sum of ion fluxes through large number of channels. Macroscopic currents can be used to obtain an estimate about the open-probability and is given by

$$I = NiP_o \tag{1}$$

that describes the total macroscopic current,I, through a homogenous population of N independent channels. Here i is the elementary current through the individual open channel and P_o represents the open probability of the channel. The single-channel current–voltage relationship is often assumed to be linear

$$i = \gamma(V - V_0) \tag{2}$$

γ is the single-channel conductance and V_0 represents the Nernst potential.

We can get Eq. (3) by combining Eqs. (1) and (2).

$$I = N\gamma P_o(V - V_0) = g_m P_o(V - V_0) \tag{3}$$

where $g_m = N\gamma$ is the maximum conductance of N independent channels. As the channel conducts Cl^-, V_0 is replaced by V_{Cl}, given by

$$V_{Cl} = RTIn([Cl^-]_i/[Cl^-]_o)/(ZF) \tag{4}$$

Z is the valence of Cl^-, R is the universal gas constant, F is the Faraday constant, T is absolute temperature and $[Cl^-]_o$ and $[Cl^-]_i$ are the extracellular and intracellular concentrations of Cl^-, respectively.

For typical voltage-gated channels, the ionic conductance is, in general, a sigmoidal function with respect to membrane voltage. For CaCCs, the voltage dependence is sigmoid at all Ca^{2+} levels and is shifted to more hyperpolarized potentials as the intracellular concentration of Ca^{2+}, $[Ca^{2+}]_i$, increases (Xiao et al., 2011; Arreola et al., 1996). It can be deduced that when $[Ca^{2+}]_i$ is near zero, P_o is totally dependent on voltage, but the channel is close in the range of physiological membrane potential. Ca^{2+} can increase P_o and the activity of CaCCs is tightly coupled to changes in $[Ca^{2+}]_i$. Therefore, we propose that the steady state open probability is given by

$$P_0 = \{1 + \exp[x_1 + x_2 + x_3 + b]\}^{-1} \tag{5}$$

where

$$x_1 = -\alpha(V - V_{Cl})/|V_{Cl}| \tag{6}$$

$$x_2 = \beta[Ca^{2+}]_0(V - V_{Cl})/[Ca^{2+}]_i|V_{Cl}| \tag{7}$$

$$x_3 = -([Ca^{2+}]_i/[Ca^{2+}]_0 - 1)^d \tag{8}$$

For CaCCs, the macroscopic currents are negligible and CaCCs cannot be activated with ≤ 10 nM$[Ca^{2+}]_i$ (Kuruma and Hartzell, 2000; Evans and Marty, 1986; Galietta, 2009; Boese et al., 2004; Xiao et al., 2011; Arreola et al., 1996). Therefore, we define $[Ca^{2+}]_0 =$ 10 nM for dimensionless. In Eq. (5), the effect of the membrane voltage on P_0 is represented by x_1, which is independent of $[Ca^{2+}]_i$. The dimensionless constant, α, describes the steep voltage dependence of P_0. The coupling effect between the membrane voltage and the intracellular Ca^{2+} on P_0 is represented by x_2, wherein the stiffness of coupling between $[Ca^{2+}]_i$ and voltage embody by β, a dimensionless constant. The effect of the intracellular Ca^{2+} on P_0 is represented by x_3, which is independent of the membrane voltage. The dimensionless constant,d, is related to the affinity of CaCCs to Ca^{2+}, for the more d is, the more the term x_3 influence P_0 at given $[Ca^{2+}]_i$. The constant b represents the channel's closed state, which is determined by the minimum conductance.

Some explanation is given below on how to get the values of the above parameters. First, within a typical cell, the intracellular concentration of free calcium is normally maintained in the range of 10–100 nM (Nakatsu et al., 2007), but is subject to increases of 10–100-fold during various cellular functions, when $[Ca^{2+}]_i$ is much larger than $[Ca^{2+}]_0$, and the CaCC's currents do not rectify and the relationship between current and voltage is linear. The slope of the current–voltage curve is represented by g_m. Second, when it is satisfied that $[Ca^{2+}]_i = [Ca^{2+}]_0$ and $V \approx V_{Cl}$, the channel is in closed state and the conductance is zero, the value of b can be obtained. Third, when $V \approx V_{Cl}$, the relationship between current and calcium ion concentration is needed to get the value of d. Fourth, when the values of g_m, b and d have been identified and when $[Ca^{2+}]_i \gg [Ca^{2+}]_0$, α can be calculated according to the current–voltage curve. Last, the value of β can be calculated by the current–voltage curves at given $[Ca^{2+}]_i$.

The equations are solved by Mathematica 5.0 (Wolfram research, USA). Origin 8.0 (OriginLab, Northampton, MA) is used for date analysis and graphics.

3. Results

3.1. Native CaCCs have universal gating mechanism

For validation, we select two examples that are different from each other in cell origin and patch clamp configuration. The first set of experimental data is from CaCCs in rat lacrimal gland, which was studied with the tight-seal whole-cell recording technique (Evans and Marty, 1986). The second set of experimental data is from CaCCs in *Xenopus* Oocytes, which were recorded with the excised inside-out patches (Kuruma and Hartzell, 2000). The parameter values are shown in Table 1. Figs. 1 and 2 show the experimental current and voltage curves and the corresponding

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