Development of the L-type CaV / BK Complex Simulator (II): estimation of the distance between the channels

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ABSTRACT

The presence, in the cell membrane, of high-conductance K^+ channels and voltage-gated Ca^{2+} channels (CaV) forming complexes has been reported. These complexes have important functions in excitable cells. The internal calcium ($[Ca^{2+}]_i$) at the mouth of the CaV channel decreases with distance and with the concentration of chelators. For the BK channel to be activated with internal Ca^{2+}, a concentration of the order of μM is necessary and this implies a closeness between the BK-CaV channels. A simulator of the decay of Ca^{2+} in the presence of BAPTA to estimate the distance between the channels was developed. The mathematical models were implemented in Visual Basic® 6.0 and were solved numerically. The

results indicate the coexistence of L-type CaV channel and BK grouped in nanodomains with a distance between channels of ~30 nm.

Keywords: Simulators, L-type CaV – BK microdomain, BK channels, L-type CaV channels.

1 INTRODUCTION

1.1 ASSOCIATION OF BK CHANNELS WITH VOLTAGE-GATED CA^{2+} CHANNELS.

Different works have been reported showing the association of high conductance voltage dependent K^+ channels (BK) and Ca^{2+} with different voltage-dependent Ca^{2+} channels (CaV), forming complexes in excitable and non-excitable cells (Guéguinou et al., 2014).

The opening of the Ca^{2+} channel produces an influx of Ca^{2+} that immediately increases the concentration at the mouth of the channel up to 50 to 100 μM in a space of tens of nanometers and decreases with distance due to the action of buffers and the diffusion process (Vivas et al., 2017). In pyramidal hippocampal neurons, BK channels are activated within 0.5 ms by Ca^{2+} that enters through the CaV channel when it is opened by the depolarization of a single action potential. This short time required for the BK channels to activate implies a closeness between the BK and CaV channels (Grunnet & Kaufmann, 2004; C. S. Müller et al., 2010; Rehak et al., 2013).

1.2 CAV/BK NANODOMAIN AND MICRODOMAIN

The BK channels are spatially close to the voltage-gated calcium channels (CaV) of the P/Q-type (Edgerton & Reinhart, 2003; Womack et al., 2004), of the N-type (Marrion & Tavalin, 1998) and L-type channels (Prakriya & Lingle, 1999). Immunoprecipitation studies indicate a coexistence of BK/L-type channels in rat brain (Grunnet & Kaufmann, 2004). L-type CaV/BK complexes are found in sympathetic and hippocampal neurons, where they regulate neuronal excitability at voltages closed to the threshold of AP (Vivas et al., 2017). The CaV/BK complex works as a negative feedback system. Calcium enters the neuron through calcium-permeable channels (CaV), the intracellular concentration of Ca^{2+} increases, activates the BK channel, the cell becomes hyperpolarized, and a negative feedback pathway of the influx of Ca^{2+} occurs through voltage-gated calcium channels (Contet et al., 2016). The influx of Ca^{2+} through the CaV channel takes place for a short period of time in an intracellular space restricted to one domain by the action of chelators that limits its diffusion (Müller et al., 2007; Naraghi & Neher, 1997). Fakler and Adelman, analyzed the decrease of $[Ca^{2+}]_i$ by BAPTA and EGTA, from a $[Ca^{2+}]$ at the mouth of the CaV channel of 20 μM (Fakler & Adelman, 2008). Augustine et al., classified calcium domains according to

the action of BAPTA or EGTA in: (1) calcium nanodomains (~20 to 50 nm from the calcium source) and (2) microdomains (~50 to about hundreds of nanometers away from the source) (Augustine et al., 2003). The calcium peak reached in nanodomains is greater than in microdomains (~100 μM versus 1-5 μM) and the duration of the calcium signals lasts microseconds in nanodomains, while it occurs in milliseconds in microdomains (Nowycky & Pinter, 1990). In very short times the Ca^{2+} concentration can reach up to ~100 μM (Rizzuto & Pozzan, 2006). In frog saccular hair cells the concentration of Ca^{2+} at the mouth of the calcium channel is >100 μM (Roberts, 1994). This high concentration is essential for Ca^{2+} to reach the receptor in a short period of time (Roberts, 1994). $[Ca^{2+}]_i$ levels are controlled by proteins that bind it (endogenous chelating molecules) (A. Müller et al., 2007; Naraghi & Neher, 1997), by the SERCA and PMCA pumps.

1.3 OBJECTIVE

To have a simulator that allows the estimation of the distance between L-type CaV channels and BK channels in neurons.

2 MATERIAL AND METHODS

A simulator was designed and developed for the BK/L-type CaV complex in the neuron soma to determine the distance between the channels.

2.1 CA^{2+} DIFFUSION SIMULATOR IN THE PRESENCE OF BAPTA (ONE-DIMENSIONAL APPROXIMATION)

To estimate the distance between the L-type CaV channel and the BK channel, the mathematical development proposed by Müller (A. Müller et al., 2007) was used. In a CaV-BK domain an increase in internal calcium occurs first as a result of ion influx through the Ca^{2+} channel (Eq. 1).

$$\Delta[Ca^{2+}] = \frac{i_{Ca}}{4\pi\, FD_{Ca} r} \quad (1)$$

And then a decrease in distance affected by BAPTA (Eqs. 2 and 3).

$$\Delta[Ca^{2+}]^{BAPTA} = \frac{i_{Ca}}{4\pi\, FD_{Ca} r} \exp\left(-\frac{r}{\lambda}\right) \quad (2)$$

$$\lambda = \sqrt{\frac{D_{Ca}}{k_{on}[BAPTA]_{free}}} \quad (3)$$

Where: i_{Ca} is current in a Ca^{2+} channel, F Faraday constant, D_{Ca} diffusion constant for Ca^{2+}, r distance from a Ca^{2+} channel, $BAPTA_{free}$ the concentration of BAPTA not bound to Ca^{2+}, k_{on} binding velocity rate to Ca^{2+}.

Dividing the Eq. 1 by the Eq. 2 gives an expression of the relative Ca^{2+} close to the BK channel in the presence of BAPTA (Eq. 4). The concentration of Ca^{2+} in the distance depends on the concentration of BAPTA.

$$^{BAPTA}[Ca^{2+}]_{BK} = \frac{\Delta[Ca^{2+}]_{BK}^{BAPTA}}{\Delta[Ca^{2+}]_{BK}} = \exp\left(-\frac{r}{\lambda}\right) \quad (4)$$

The simulator allows $[Ca^{2+}]$ to enter the mouth of the L-type CaV channel according to the data reported (Brenner et al., 2000; Fakler & Adelman, 2008) and the distance to the BK channel is estimated when $^{BAPTA}[Ca^{2+}]_{BK}$ = ~10 μM (Brenner et al., 2000; Contreras et al., 2013; Cox, 2014). The parameters used are: $[Ca^{2+}]_i$ = 0.05 μM (at rest), $BAPTA_{free}$ 3000 μM, D_{Ca} = 220 μM/s, k_{on} = 4x10^8 $M^{-1}s^{-1}$ (Naraghi & Neher, 1997).

The mathematical models were implemented with the Visual Basic 6.0 language for Widnows evironment.

3 RESULTS

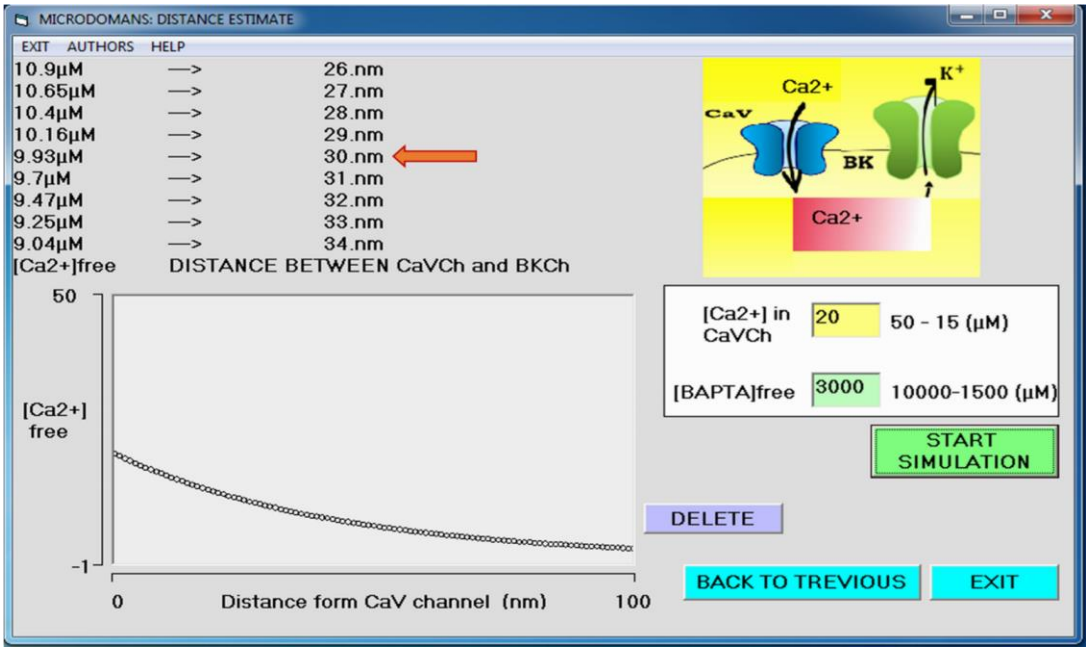
3.1 ESTIMATION OF THE DISTANCE OF THE L-TYPE CAV / BK COMPLEX

To estimate the distance between the L-type CaV channel and BK, a simulator was developed (Figure 1). The interface has a data entry module: (1) $[Ca^{2+}]_i$ located immediately in the CaV channel and (2) free concentration of BAPTA. It has a box to graph the diffusion of Ca^{2+} with respect to distance.

With the depolarization of the membrane, the calcium channel is activated by voltage and an influx of Ca^{2+} into the cytoplasm begins. The Ca^{2+} concentration is of the order of μM immediately to the CaV channel (Brenner et al., 2000). Fakler and Adelman propose a concentration at that point of 20 μM (Fakler & Adelman, 2008). The concentration of $[Ca^{2+}]_i$ in resting state is ~50 to 100 nM (Foskett et al., 2007). A concentration difference is established between 20 μM and 50 nM, and the diffusion process begins. BAPTA and EGTA bind Ca^{2+}. According to Naraghi and Neher, the free concentration of BAPTA is 3 mM (Naraghi & Neher, 1997). The Ca^{2+} concentration decreases with distance. Depending on the

concentration of BAPTA, the distance reached by Ca^{2+} may decrease. A simulation was performed considering the data from Fakler and Adelman (2008) and Naraghi and Neher (1997) as physiological conditions and the distance where $[Ca^{2+}]_i$ decreases ~10 μM (concentration necessary to activate the BK channel) was estimated (Fakler & Adelman, 2008; Naraghi & Neher, 1997). Under these conditions, the results of the colocalization of the L-type CaV channel with the BK channel would have a distance of ~ 30 nm (Figure 1). The L-type CaV / BK complex would be a nanodomain. These results are similar to the colocalization of BK channels with CaV2.1 (P/Q) channels within approximately 40 nm (Indriati et al., 2013; Takahashi & Wood, 1970). This suggests that the CaV2.1 channel clustered with BK also forms nanodomains. Other authors estimate a distance between CaV-BK channels of ~10 to 15 nm in hippocampal granule cells (Cox, 2014; A. Müller et al., 2007). These short distances suggest a spatial arrangement of the channels that cannot be explained by a random distribution (A. Müller et al., 2007).

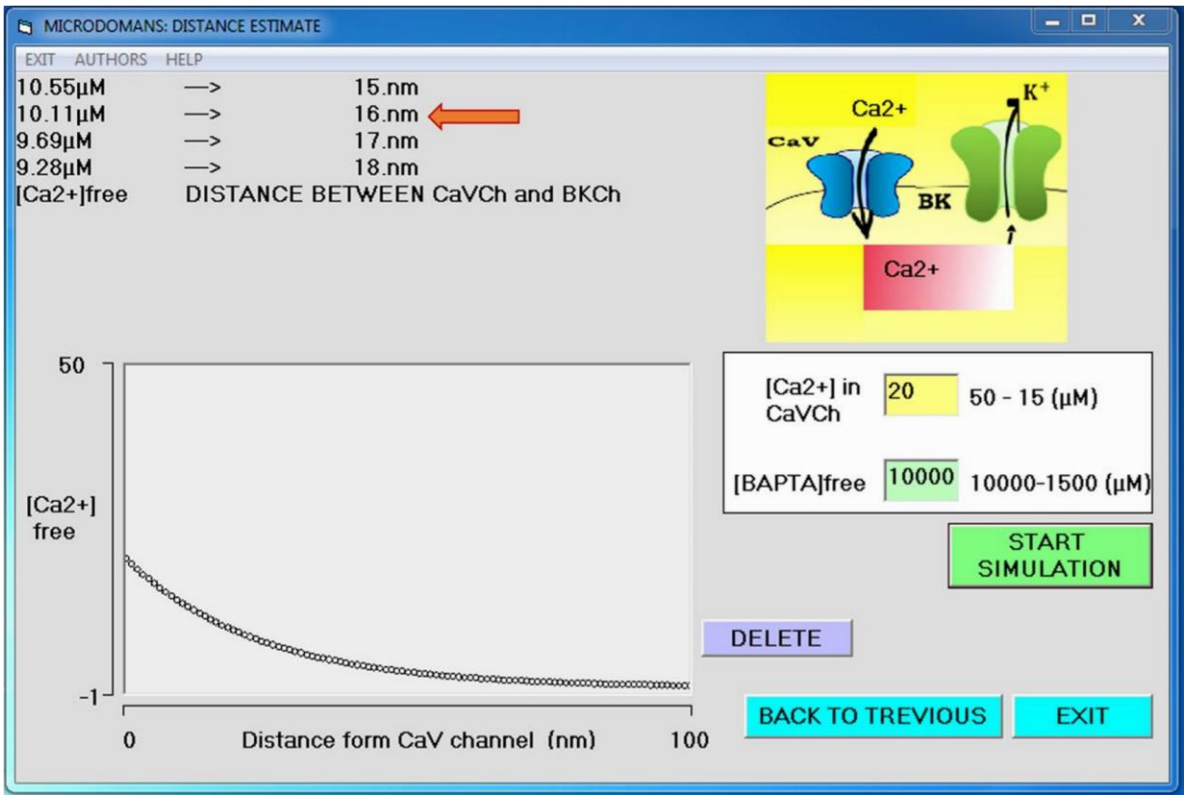
Figure 1. Simulations to estimate the distance between the BK channel and L-type CaV. The simulator shows the graph of the decay of the internal Ca^{2+} concentration. In the upper left, Ca^{2+} concentrations are presented at different distances. The arrow indicates the concentration closest to 10 μM and the estimated distance. Simulation with $[Ca^{2+}]_i$ = 20 μM at the mouth of the CaV channel and $[BAPTA]_{free}$ = 3 mM. The estimated distance was ~30 nm (arrow in red).



In this simulator it can be observed that the calculated distance between channels is determined by the Ca^{2+} concentration at the mouth of the CaV channel (determined by the type of CaV channel, the membrane voltage and the difference in internal and external Ca^{2+} concentrations) and by the type of Ca^{2+} buffer (BAPTA, EGTA, calbidin) (its diffusion constant and its concentration). Concentrations >5 mM of

BAPTA interfere in the activation of the BK channel due to the distance where the BK channel is located, the $[Ca^{2+}]_i$ is not enough to activate it (Fakler & Adelman, 2008). A BAPTA concentration of 10 mM causes a faster decay of $[Ca^{2+}]_i$ and the distance where ~10 μM is reached was 16 nm (Figure 2). Consequently, not enough Ca^{2+} reaches the BK channel (calculated at ~30 nm).

Figure 2. Simulation with increase of free [BAPTA] = 10 mM. The decay of the Ca^{2+} concentration is faster. The estimated distance was ~16 nm.



4 CONCLUSIONS

The results of the simulations suggest an interaction between the L-type CaV and BK channels in complexes that form possible nanodomains. The high concentrations of internal Ca^{2+} (μM) achieved to activate the BK channel would be possible as long as the BK and L-type CaV channels are placed in very small spaces (<40 nm).

For other conditions, if the researcher has data of $[Ca^{2+}]$ at the mouth of the CaV channel and of the necessary $[Ca^{2+}]$ to activate the BK channel, with this simulator he will be able to estimate the distance between these channels. This simulator can be used as a teaching tool in postgraduate physiology and biophysics courses.

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