Theoretical Study of the Function of the IP3 Receptor/BK Channel Complex in a Single Neuron

Estudio Teórico de la Función del Complejo Receptor-IP3/Canales BK en Neurona Única

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ABSTRACT

Large conductance calcium-activated potassium (BK) channels carry out many functions in the central nervous system. These channels open in response to increased cytosolic calcium ([Ca$^{2+}$]$_{cyt}$) concentration. The influx of calcium ions to the cytosol can occur through voltage-gated calcium channels (VGCCs) on the plasma membrane and/or through IP$_3$ receptors (IP$_3$-Rs) and ryanodine receptors (RyRs) on the endoplasmic reticulum membrane. The BK channel/IP$_3$-R/RyR interaction has been widely reported in smooth muscle but scarcely investigated in relation to neurons. The aim of this study was to theoretically explore the function of the BK/IP$_3$-R complex by means of a computational model of a neuron that replicates the interaction between the release of Ca$^{2+}$ from the endoplasmic reticulum (through IP$_3$-Rs) and the opening of the BK channels. The mathematical models are based on the Hodgkin-Huxley formalism and the Goldbeter model. These models were implemented on Visual Basic® and differential equations were solved numerically. Distinct conditions were contemplated for BK conductance and the efflux of endoplasmic Ca$^{2+}$ to the cytosol. An abrupt rise in [Ca$^{2+}$]$_{cyt}$ ($\geq$ 5 $\mu$M) and short duration (spark) was found to activate BK channels and either pause or stop the action potential train.

KEYWORDS: BK channels; IP3 receptor; Ca$^{2+}$ microdomains; Single-neuron simulations; SERCA pump
RESUMEN
Los canales de potasio activados por calcio de gran conductancia (canales BK) cumplen múltiples funciones en el sistema nervioso central. Estos canales se abren en respuesta al incremento de la concentración de calcio citosólico ([Ca\(^{2+}\)\(_{\text{cyt}}\)]. La entrada de Ca\(^{2+}\) puede ocurrir a través de canales de calcio dependientes de voltaje (VGCCs) localizados en la membrana plasmática y por eflujo de Ca\(^{2+}\) del retículo endoplásmico (ER) causado por 1,4,5-Trifosfato (IP\(_3\)) o rianodina (RyR). La interacción BK/IP\(_3\)/RyR ha sido ampliamente estudiada en músculo liso, pero escasamente en neuronas. El objetivo de este estudio fue explorar teóricamente la función del complejo BK/IP\(_3\)-R mediante un modelo computacional de una neurona que replica la interacción entre la liberación de Ca\(^{2+}\) del retículo endoplásmico (a través de IP\(_3\)-Rs) y la apertura de los canales BK. Los modelos matemáticos se basan en el formalismo de Hodgkin-Huxley y el modelo de Goldbeter. Estos modelos fueron implementados en Visual Basic\textsuperscript® y las ecuaciones diferenciales fueron resueltas por métodos numéricos. Se contemplaron distintas condiciones para la conductancia del canal BK y la salida de Ca\(^{2+}\) endoplásmico al citosol. Los resultados muestran que un incremento abrupto de [Ca\(^{2+}\)\(_{\text{cyt}}\) (≥ 5 µM) y de corta duración (spark) activa los canales BK y producen una pausa o detiene el tren de potenciales de acción.

PALABRAS CLAVE: BK channels; IP3 receptor; Ca\(^{2+}\) microdomains; Single-neuron simulations; SERCA pump

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INTRODUCTION

In neurons, maintaining the homeostasis of calcium ions (Ca\(^{2+}\)) is essential for proper signaling and function. The endoplasmic reticulum (ER) serves as the main Ca\(^{2+}\) storage organelle in neurons and has mechanisms of influx and efflux of this ion. Such mechanisms, located on the ER membrane, help to regulate the cytosolic concentration of Ca\(^{2+}\). The influx mechanism is based on Ca\(^{2+}\) pumps, while the most important efflux mechanisms are triggered by inositol 1,4,5-trisphosphate (IP\(_3\)) receptors (IP\(_3\)-Rs) and ryanodine receptors (RyRs). The rise in the level of Ca\(^{2+}\) in the cytosol activates the large-conductance calcium-activated potassium channels, or big potassium (BK) channels.

Various mathematical models have been developed to replicate the release of Ca\(^{2+}\) from the ER and the pattern of the cytosolic Ca\(^{2+}\) concentration, hereafter denominated [Ca\(^{2+}\)]\(_{cyt}\). However, they have not been associated with BK channels in order to research their joint function at the somatic level, explore the mechanisms involved, and determine the importance they have in neuronal activity. A theoretical study was carried out on the functional coupling of IP\(_3\)-Rs to BK channels in a single neuron and the effects produced. An interaction is proposed in the soma of the neuron in a microdomain formed by the proximity of the BK channels of the plasma membrane to the calcium efflux channels triggered by the IP\(_3\)-Rs located on the ER membrane.

BK channels

Large conductance (BK) channels are part of the family of calcium-activated potassium channels. They are distinguished from other calcium-activated potassium channels (small and intermediate conductance) by their capacity to couple [Ca\(^{2+}\)]\(_{cyt}\) and sense the membrane potential variations. BK channels are widespread in the brain, being found in the cerebral cortices, cerebellar cortex, hippocampus, olfactory bulb, vestibular nuclei, basal ganglia, hypothalamus, and thalamus [1]. Calcium-activated potassium channels exist in diverse cell types [2]. On neurons, BK channels are present in dendrites, soma, axons, and synaptic terminals [3]. Upon analyzing aplysia nerve cells, Meech [3] identified for the first time a potassium current activated by a rise in the level of [Ca\(^{2+}\)]\(_{cyt}\). After microinjection of calcium, the cell membrane was hyperpolarized and an increase in Ca\(^{2+}\)-activated K\(^+\) conductance (gK\(_{Ca}\)) occurred concomitantly [3]. Since then, these channels have been investigated to characterize their molecular structure, electrophysiology, and pharmacology [4]. According to their single-channel conductance, K\(_{Ca}\) channels are divided into three main subfamilies: (1) small conductance (SK) (4-14 pS), (2) intermediate conductance (IK) (32-39 pS), and (3) large conductance (BK) (200-300 pS) channels [4][5].

The BK channels have several distinctive characteristics. (1) They are homotetramers with two regulatory domains containing two high-affinity Ca\(^{2+}\) binding sites. (2) They are voltage and calcium-dependent, requiring both membrane depolarization and calcium for their activation. Ca\(^{2+}\) binding and voltage sensor activation act almost independently to enhance channel opening [6]. BK channels can open in the absence of calcium but are more sensitive to calcium at depolarizing voltage steps [5]. Hence, their sensitivity to calcium is strongly dependent on the membrane potential. The dissociation constant (Kd) for calcium is in the micromolar range at -60 mV and in the nanomolar range at +20 to +40 mV [7]. (3) BK channels are fast activating (on the order of 1 ms or less) compared to IK and SK channels, which have a slow activation time (lasting hundreds of milliseconds or over a second, respectively) [8].

BK channels have various functions in the central nervous system. At the soma of many neuronal cells, they control the speed of action potential repolarization and mediate the rapidity of afterhyperpolarization. Therefore, they can influence spike frequency adaptation [9][10]. They are often physically associated...
with voltage-gated calcium channels (VGCCs), thus forming microdomains with them. Due to their presence in nerve terminals and their co-assembly with VGCCs at active zones, BK channels are particularly suitable for regulating the release of neurotransmitters, increase the duration of PA, prevent back-propagation in dendrites, and produce a decrease in firing frequency.

The concentration of free and bound \( [\text{Ca}^{2+}]_{\text{cyt}} \) is reported to be approximately 100 nM and 10 μM, respectively. While a localized increase in \( [\text{Ca}^{2+}]_{\text{cyt}} \) has been evidenced in some studies, others show spatio-temporal calcium signaling restricted to nano and microdomains in neurons and smooth muscle.

In a BK channel-VGCC microdomain, according to Fakler and Adelman, the kinetics of \( [\text{Ca}^{2+}]_{\text{cyt}} \) exhibit a localized boost of up to 20 μM in neurons. The \( [\text{Ca}^{2+}]_{\text{cyt}} \) concentration decreases with distance due to the diffusion process and interaction with the chelators (1,2-bis(o-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid (BAPTA) and ethylene glycol-bis(b-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA).

**Endoplasmic reticulum (ER)**

Because of containing a high concentration of \( \text{Ca}^{2+} \)-binding proteins, the ER is the main \( \text{Ca}^{2+} \) storage organelle in cells. Indeed, the total amount of \( \text{Ca}^{2+} \) may be >1 mM, while the concentration of free \( [\text{Ca}^{2+}]_{\text{ER}} \) (\( \text{Ca}^{2+} \) in the ER) has been quantified at 100-700 μM.

To maintain equilibrium, mechanisms of influx and efflux of \( \text{Ca}^{2+} \) are activated on the ER membrane. There are two types of processes related to the efflux of \( \text{Ca}^{2+} \) from the ER, being the \( \text{Ca}^{2+} \)-induced \( \text{Ca}^{2+} \) release (CICR) and IP\(_3\)-induced \( \text{Ca}^{2+} \) release (IIICR) processes. The \( [\text{Ca}^{2+}]_{\text{cyt}} \) interacts with ryanodine receptors and IP\(_3\) receptors (RyRs, IP\(_3\)-Rs) in the former and IP\(_3\) with its receptors (IP\(_3\)-Rs) in the latter, in both cases to release \( \text{Ca}^{2+} \) from the ER. On the other hand, \( \text{Ca}^{2+} \) is recaptured into the ER by the activity of smooth endoplasmic reticulum \( \text{Ca}^{2+}\text{-ATPase (SERCA) pumps} \). Thus, a low concentration of \( [\text{Ca}^{2+}]_{\text{cyt}} \) (50 - 100 nM) is maintained by the coordinated action of the inflow of \( \text{Ca}^{2+} \) to the ER through pumps on the ER membrane, and the efflux of \( \text{Ca}^{2+} \) from the cytosol to the extracellular space through pumps (PMCA) on the plasma membrane.

**BK channel – IP\(_3\) receptor interaction**

The BK channel/IP\(_3\)-R microdomain has received less attention, and its role is controversial. IP\(_3\)-Rs are localized in the ER membrane and the BK channels in the plasma membrane. The BK channels and IP\(_3\) receptors are very close to one another. The ER membrane is believed to be initially generated as part of the nuclear envelope, which then expands and morphs into a complex reticulum that can extend to distant cellular compartments such as the axons, dendrites, and dendritic spines of neurons, but with a similar morphology and closeness between the ER membrane and the cytoplasmic membrane. The cisternae of the ER are classified in accordance with their proximity to the plasma membrane. Type I is the farthest from the plasma membrane, while type II and III are nearer, frequently following its profile.

Pan et al., reported the interaction between BK channels and IP\(_3\)-Rs in human embryonic kidney cells (HEK293). Neuropeptide galanin activates galanin receptors (GalR2s), and IP\(_3\)-Rs are activated through the protein kinase G pathway. The increase in \( [\text{Ca}^{2+}]_{\text{cyt}} \) is due to \( \text{Ca}^{2+} \) efflux from the ER through IP\(_3\)-Rs. The authors demonstrated that the rise in the level of \( [\text{Ca}^{2+}]_{\text{cyt}} \) comes from the ER but did not quantify this change.

In arterial smooth muscle cells, relaxation and contraction are regulated by calcium released from the sarcoplasmic reticulum. The flow of calcium from the ER to the cytosol (induced by IP\(_3\) and ryanodine) activates the BK channels, thus facilitating a negative feedback mechanism in opposition to vasoconstric-
There is evidence of the proximity of BK channels and calcium release sites. This is further supported by co-immunoprecipitation experiments \[28\]. As a consequence, such channels would be exposed to a high calcium concentration (>10 μM, in the order of 1-100 μM).

In neurons, Irie and Trussell \[23\] described a nanodomain between RyRs on the ER membrane and plasma membrane VGCCs (voltage-gated Ca\(^{2+}\) channels), and another one between RyRs and BK channels in the soma of cartwheel inhibitory interneurons of the dorsal cochlear nucleus. Through the VGCC-RyR interaction, the latter receptors trigger the release of Ca\(^{2+}\). The internal increase in calcium acts on plasma membrane BK channels to control action potential activity and shape the burst. The interaction of the nanodomains and the Ca\(^{2+}\) transients must be very rapid (in a millisecond timescale), and thus arise only tens of nanometers from the plasma membrane \[23\].

\( \text{IP}_3 \) is highly mobile in the cytosol. It is synthesized in the plasma membrane and diffuses into the cell where it encounters its specific receptors (\( \text{IP}_3 \)-Rs) on the ER \[29\]. In neocortical pyramidal neurons, \( \text{IP}_3 \) produces calcium waves via activation of metabotropic glutamate receptors. When measured with non-buffering low-affinity Ca\(^{2+}\) indicators, such waves have a peak amplitude of over 5 μM \[30\] and propagate with a velocity of ~100 μm/s \[31\]. According to Ross \[31\], the release of Ca\(^{2+}\) from ER has been less studied because it is not associated with specific changes in the membrane potential. As can be seen in this work, the impact of the release of Ca\(^{2+}\) from ER on the membrane potential was researched indirectly through the BK channels.

The importance of the interaction between BK channels and voltage-gated calcium channels has been demonstrated in the release of neurotransmitters, where they play a regulatory role that prevents excitotoxicity \[32\]; in the smooth muscle of blood vessels, where it regulates blood pressure and plays an important role in preventing hypertension \[33\]. In these cases, the prevention mechanism is a negative feedback system. The experimental study is facilitated because both channels are found on the same membrane, and voltage clamping and transfections can be performed to combine different types of VGCC channels with the BK channel, etc. On the other hand, the experimental study of the BK/IP\(_3\)-R complex is more difficult, the channels are in different membranes and consequently, voltage clamping cannot be performed. This justifies a theoretical study of the BK/IP\(_3\)-R. It is unknown whether, at a somatic level in neurons, the BK/IP\(_3\)-R interaction is present as a protective mechanism. Based on the reported studies, the coexistence of BK and IP\(_3\)-R channels at the somatic level is known \[23\] and of the proximity of the cell membrane and the endoplasmic reticulum and contact sites between these structures \[24\], necessary conditions for the presence of BK/IP\(_3\)-R. The proposed hypothesis is that: in neurons, at the somatic level, there is a BK/IP\(_3\)-R interaction. If this is so, what would its role be in neuronal activity? What would be the mechanisms involved? It is not known whether an abrupt and short-term outflow of Ca\(^{2+}\) could activate BK channels in the neuron’s soma; it is also not known whether there is a commitment between the number of BK channels present in the membrane and the response of the neuron to [Ca\(^{2+}\)]\(_{\text{cyt}}\).

**Modeling of Ca\(^{2+}\) release from IP\(_3\) R**

Based on detailed knowledge of the timescales of Ca\(^{2+}\) release from the ER and the pattern of [Ca\(^{2+}\)]\(_{\text{cyt}}\) concentration, mathematical models have been developed to replicate variations in [Ca\(^{2+}\)]\(_{\text{cyt}}\). The resulting models are of three types. Firstly, there are models for the release of Ca\(^{2+}\) (with simplified dynamics) from the ER or the sarcoplasmic reticulum through the IP\(_3\)-R. Such early models are purely phenomenological \[34\] \[35\]. Secondly, some models incorporate molecular details of the interaction of IP\(_3\) and Ca\(^{2+}\) with the IP\(_3\)-R, consid-
ering microscopic kinetics and a detailed gating scheme for the receptor \cite{36,37,38,39,40,41}. Thirdly, some models (e.g., the one created by Blackwell and Kotaliski, in \cite{39}) also incorporate second messengers within the biochemical reactions that are triggered by metabotropic glutamate receptor (mGluR) activation and lead to IP$_3$ production. Each group of models has different timescales.

The mathematical models are related to the molecular interaction of IP$_3$ with the IP$_3$-R and the Ca$^{2+}$-activated channel, which are activated sequentially for the release of Ca$^{2+}$ from the ER. These models have employed different mathematical techniques \cite{40}. The current contribution focuses on the interaction of [Ca$^{2+}$]$_{\text{ER}}$ with BK channels. Detailed molecular kinetics of the mechanisms of release of Ca$^{2+}$ from the ER is not essential for the purpose of this work. The phenomenological model of Goldbeter of such Ca$^{2+}$ release was herein found to be sufficiently accurate and appropriate in its timescale and was combined with the phenomenological model of Hodgkin and Huxley. The latter formulation describes the electrical activity of neurons \cite{41}. Based on the aforementioned models, the present study theoretically explored the function of a BK/IP$_3$-R microdomain.

**MATERIALS AND METHODS**

In the development of a new model, consideration was given to the spatial structure of the soma and some key concepts related to the kinetics of Ca$^{2+}$. The increase in Ca$^{2+}$ is located in the microdomain \cite{16} formed mainly by the following factors \cite{28}: the BK channels on the soma membrane \cite{11,44} and the Ca$^{2+}$- and IP$_3$-R-sensitive channels on the ER membrane \cite{42}. The two membranes are very close to one another \cite{26}. There is evidence from electron microscopy, with 3D reconstructions, of the proximity of the plasmatic membrane with the ER membrane and of numerous contact sites between these structures, mainly in the neuron soma \cite{24}.

**The Hodgkin and Huxley formalism and the BK model**

The electrical activity of the neuron was reproduced with the Hodgkin and Huxley formalism (H-H model), consisting of an equation that represents the membrane potential (Equation 1) and others that define channel gating variables (Equations 2-5) \cite{45}.

$$\frac{dV}{dt} = -\bar{g}_m m^3 h (V - E_{Na}) - \bar{g}_n n^4 (V - E_K) - \bar{g}_L (V - E_L) - \bar{g}_{BK} q (V - E_K) \tag{1}$$

$$\frac{dm}{dt} = \alpha_m(V)(1 - m) - \beta_m(V)m \tag{2}$$

$$\frac{dh}{dt} = \alpha_h(V)(1 - h) - \beta_h(V)h \tag{3}$$

$$\frac{dn}{dt} = \alpha_n(V)(1 - n) - \beta_n(V)n \tag{4}$$

$$\frac{dq}{dt} = \alpha_q(V)(1 - q) - \beta_q(V)q \tag{5}$$

Where $\alpha_m(V)$, $\beta_m(V)$, $\alpha_n(V)$, $\beta_n(V)$, $\alpha_q(V)$, $\beta_q(V)$, $\alpha_q(V)$, and $\beta_q(V)$ are activation and inactivation gating variables and are defined according to the following empirical equations (Equations 6-13) \cite{45}.

$$\alpha_m(V) = \frac{-0.1(V+40)}{1 - \exp\left(-\frac{V+40}{10}\right)} \tag{6}$$

$$\beta_m(V) = 4 \exp\left(-\frac{V+65}{18}\right) \tag{7}$$

$$\alpha_n(V) = 0.07 \exp\left(-\frac{V+65}{20}\right) \tag{8}$$

$$\beta_n(V) = \frac{1}{1 + \exp\left(-\frac{V+30}{10}\right)} \tag{9}$$
\[ \alpha_n(V) = \frac{0.01(V+55)}{1-\exp\left(-\frac{V+65}{80}\right)} \]  
\[ \beta_n(V) = 0.125 \exp\left(-\frac{(V+65)}{80}\right) \]  
\[ \alpha_q(V) = e^{(V/27)} \frac{200-[Ca^{2+}]_{\text{cyt}}}{\exp\left(\frac{200-[Ca^{2+}]_{\text{cyt}}}{20}\right)-1} \]  
\[ \beta_q(V) = 0.0004 \text{ to } 0.002 \]

**Table 1. The parameters of the H-H model (from [45]).**

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Values</th>
<th>Units</th>
<th>Definitions</th>
</tr>
</thead>
<tbody>
<tr>
<td>V</td>
<td>-90 *</td>
<td>mV</td>
<td>Membrane potential</td>
</tr>
<tr>
<td>Cm</td>
<td>1</td>
<td>µF cm(^{-2})</td>
<td>Membrane capacitance</td>
</tr>
<tr>
<td>E_{mem}</td>
<td>50</td>
<td>mV</td>
<td>Equilibrium potential for Na(^{+}), K(^{+}), and leak</td>
</tr>
<tr>
<td>E_{k}</td>
<td>-77</td>
<td>mV</td>
<td>Maximum conductance for Na(^{+}), K(^{+}), and leak and BK</td>
</tr>
<tr>
<td>(\bar{g}_{m})</td>
<td>120</td>
<td>mS cm(^{-2})</td>
<td>Membrane conductance for Ca (^{2+})</td>
</tr>
<tr>
<td>(\bar{g}_{k})</td>
<td>20</td>
<td>mS cm(^{-2})</td>
<td>Membrane conductance for Ca (^{2+})</td>
</tr>
<tr>
<td>(\bar{g}_{l})</td>
<td>0.3</td>
<td>mS cm(^{-2})</td>
<td>Membrane conductance for Ca (^{2+})</td>
</tr>
<tr>
<td>(\bar{g}_{m})</td>
<td>**</td>
<td>mS cm(^{-2})</td>
<td>Membrane conductance for Ca (^{2+})</td>
</tr>
<tr>
<td>[Ca(^{2+})](_{\text{cyt}})</td>
<td>***</td>
<td>µM</td>
<td>Calcium ions in the cytosol</td>
</tr>
</tbody>
</table>

*Initial condition
**Input into the simulator
***Is taken from the Goldbeter model

**Goldbeter Model**

The mathematical model of Goldbeter (1990) was found to be adequate [32] because the time involved in the BK channel is on a millisecond scale [48] [49] [50] and our interest is in the [Ca\(^{2+}\)]\(_{\text{cyt}}\) concentration and not in the details of the interaction of IP\(_3\) with IP\(_3\)-Rs. Hence, two different mechanisms were contemplated for the release of Ca\(^{2+}\) from the ER, one sensitive to IP\(_3\) (the IICR) [38], and the other to Ca\(^{2+}\) (the CICR) [48].

Dupont and Goldbeter developed another model with a single compartment. In a single group, considering the existence of the same two types of channels (one sensitive to Ca\(^{2+}\) and the other to IP\(_3\), no oscillations occur unless the contribution of the IICR is insignificant compared to that of the CICR [49]. Oscillations in [Ca\(^{2+}\)]\(_{\text{cyt}}\) are similar in the one- and two-compartment models [49]. The latter was employed in the present study since it does not require such an extreme decrease in the contribution of the IICR as needed in the one-compartment model.

The model has two variables, free [Ca\(^{2+}\)]\(_{\text{cyt}}\) (Z) and free [Ca\(^{2+}\)]\(_{\text{ER}}\) concentrations, modulated through the Ca\(^{2+}\) -sensitive mechanism (Y). The system is governed by the two kinetic equations [32]:

\[ \frac{dZ}{dt} = V_0 + V_1 \beta - V_2 + V_3 + K_T Y - k Z \]  
\[ \frac{dY}{dt} = V_2 - V_3 - k_T Y \]

Where \(V_0\) is related to the flow of Ca\(^{2+}\) into the cell, k to its flow out of the cell, \(V_1\) to its flow into the cytosol from the IP\(_3\)-sensitive mechanism, \(\beta\) to the saturation function of IP\(_3\)-Rs (cooperative nature), and \(K_T\) to the leaky transport of Y into Z. Finally, \(V_2\) expresses the rate of the SERCA pump and \(V_3\) the rate of transport of Ca\(^{2+}\) released from the IP\(_3\)-sensitive mechanism into the cytosol.

\[ V_2 = V_{m2} \frac{2^n_{\text{Hill}}}{K_{2}^{n_{\text{Hill}}} + 2^{n_{\text{Hill}}}} \]  
\[ V_3 = V_{m3} \frac{Y^{m_{\text{Hill}}}}{K_{R}^{m_{\text{Hill}} + Y^{m_{\text{Hill}}}} + 2^{p_{\text{Hill}}}} \]

\(V_{m2}\) denotes the maximum rate at which Ca\(^{2+}\) is pumped into the ER store and \(V_{m3}\) the IP\(_3\) -sensitive release of ions from this location. The complementary processes are represented by Hill functions. Thus, \(n_{\text{Hill}}\) and \(m_{\text{Hill}}\) depict the cooperativity coefficients, \(p_{\text{Hill}}\) reflects the degree of cooperativity of activation, and the constants \(K_r\), \(K_p\), and \(K_A\) designate the point at
which pumping, release, and activation are triggered \[49\]. Since the ATPase pump (SERCA) binds to two calcium ions per molecule of ATP, its activity is expressed by using Michaelis–Menten kinetics and a value of 2 for the Hill coefficient (Equation 16) \[50\].

Firstly, the influx of Ca\(^{2+}\) to the cytosol depends on the rate of transport through the IP\(_3\)-sensitive mechanism \((r = V_1 \cdot \beta)\) and the Ca\(^{2+}\)-sensitive mechanism \((V_3)\), the maximum rate of Ca\(^{2+}\) pumping release from the ER store \((V_{M2})\), the threshold constants for release and activation \((K_e\) and \(K_a\), respectively \((\text{Figures 1 and 5)}\)\), and the passive leak \((k_r)\). Secondly, the efflux of Ca\(^{2+}\) out of the cytosol depends on the outflow of Ca\(^{2+}\) from the cell \((k)\) (due to the PMCA pump), the inflow rate of Ca\(^{2+}\) into the ER from the cytosol, involving the rate and maximum rate of the SERCA pump \((V_2\) and \(V_{M1}\)), and the threshold constant for the latter pumping action \((K_p)\) (\(\text{Figures 1 and 7)}\). Thirdly, variables must consider the cooperativity degree of the activation process. The range of values for free \([\text{Ca}^{2+}]_{\text{cyt}}\) is 0.05 to 0.1, and that for free \([\text{Ca}^{2+}]_{\text{ER}}\) is 100 to 700 \(\mu\text{M}\) \[53\].

**RESULTS AND DISCUSSION**

The system of equations was solved simultaneously by the numerical method with a fourth-order Runge-Kutta algorithm \((dt = 0.01)\) written on Visual Basic \[53\] \[54\]. The initial conditions were \(V_1 = -90\) mV, and \([\text{Ca}^{2+}]_{\text{cyt}} = 0.1\) \(\mu\text{M}\) (value entered in the simulator). The simulator was developed and compiled to use in the Windows® environment.

When opening the simulator, the default values for each variable appear. The BK channel conductance value starts at zero. The value of free \([\text{Ca}^{2+}]_{\text{cyt}}\) and free \([\text{Ca}^{2+}]_{\text{ER}}\) are 0.1 and 100 to 700 \(\mu\text{M}\), respectively. Each simulation was carried out with the current clamp protocol. To match the timescales of the ion channels in the model with those of the Goldbeter model, a long depolarizing current pulse \((1.5\) s\) is used. Upon start-

### TABLE 2. Parameters of the Goldbeter model \[32\].

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Values</th>
<th>Units</th>
<th>Definitions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rate of transport from (\text{Ca}^{2+})-sensitive pool into the cytosol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(V_1)</td>
<td>flow rate of (\text{Ca}^{2+}) from the ER to the cytosol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(V_{M1})</td>
<td>500</td>
<td>(\mu\text{M/s})</td>
<td>maximum (V_1)</td>
</tr>
<tr>
<td>(K_k)</td>
<td>2</td>
<td>(\mu\text{M})</td>
<td>constants</td>
</tr>
<tr>
<td>(K_a)</td>
<td>.09</td>
<td>(\mu\text{M})</td>
<td></td>
</tr>
<tr>
<td>(K_r)</td>
<td>1</td>
<td>1/s</td>
<td>rate constant ((\text{Ca}^{2+}) active)</td>
</tr>
<tr>
<td>ATP-driven pumping of (\text{Ca}^{2+}) from the cytosol into the (\text{Ca}^{2+})-sensitive pool</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(V_2)</td>
<td>pumping rate of (\text{Ca}^{2+}) into the ER (by SERCA)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(V_{M2})</td>
<td>65</td>
<td>(\mu\text{M/s})</td>
<td>maximum (V_2)</td>
</tr>
<tr>
<td>(K_s)</td>
<td>1</td>
<td>(\mu\text{M})</td>
<td>constants</td>
</tr>
<tr>
<td>Efflux of (\text{Ca}^{2+}) out of the cell (PMCA bomb)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(K)</td>
<td>10</td>
<td>1/s</td>
<td>rate constant</td>
</tr>
<tr>
<td>Hill coefficients</td>
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<tr>
<td>(n_{\text{Hill}})</td>
<td>2</td>
<td></td>
<td>Hill coefficient (SERCA bomb)</td>
</tr>
<tr>
<td>(m_{\text{Hill}})</td>
<td>2</td>
<td></td>
<td>Hill coefficients (\text{Ca}^{2+}) release from (\text{Ca}^{2+})-sensitive pool</td>
</tr>
<tr>
<td>(p_{\text{Hill}})</td>
<td>4</td>
<td></td>
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</tr>
</tbody>
</table>

The default values for each variable are set at zero. The value of free \([\text{Ca}^{2+}]_{\text{cyt}}\) and free \([\text{Ca}^{2+}]_{\text{ER}}\) are 0.1 and 100 to 700 \(\mu\text{M}\), respectively. Each simulation was carried out with the current clamp protocol. To match the timescales of the ion channels in the model with those of the Goldbeter model, a long depolarizing current pulse \((1.5\) s\) is used. Upon start-
ing a simulation under these conditions, the recorded action potentials correspond to the Hodgkin and Huxley model with only voltage-dependent Na⁺ and K⁺ channels. The parameters of the Goldbeter model leading to oscillations in Ca²⁺ begin at the reference values, and immediately after there is an increase in [Ca²⁺]_{cyt} of 5 μM with a duration of 96 ms, (a local [Ca²⁺]_{cyt} transient) followed by calcium spikes of 741 nM (Figure 2).

![FIGURE 2. Electrophysiology interface simulator with three oscilloscope screens. In the control simulation, the spike train generated by the Hodgkin and Huxley model is displayed on the upper screen. The local cytosolic Ca²⁺ transient followed by three Ca²⁺ spikes is exhibited on the middle screen. The stimulus current pulse (with a duration of 1500 ms) is illustrated on the bottom screen. In this simulation, the BK conductance is zero.](image)

Khodakhah and Ogden [54] reported that IP₃ triggers a release of Ca²⁺ from the ER with an initial well-defined delay, which decreases as the concentration of IP₃ rises (mean, 85 ms at 10 μM IP₃). In the present study, the simulation is initiated at the moment Ca²⁺ is released from the ER. Hence, a series of factors are not considered in the model: the interaction of the first messenger with the receptor on the plasma membrane, the cascade of second messengers, the explicit interaction of IP₃ and Ca²⁺ with IP₃-Rs, and the corresponding delay in the release of Ca²⁺. The dynamics of Ca²⁺ is local. The diffusion and chelation of Ca²⁺ are not simulated.

Diverse physiological processes (including pathological phenomena) are modulated by the generation and propagation of [Ca²⁺]_{cyt} signals. The release of Ca²⁺ from the ER takes place through sparklets, sparks, blink, scintilla, puffs, and other forms [16]. Since such elementary events are produced in microdomains, the multiple forms of Ca²⁺ release confer intracellular Ca²⁺ signaling with a broad architecture in space, time, and intensity, which in turn underlies signaling efficiency, stability, specificity, and diversity [55]. The calcium buffers are instrumental in achieving temporal, spatial, and functional compartmentalization under these conditions, creating steep gradients in a close proximity of channels, until reaching an internal calcium concentration on the order of tens of micromoles [56]. The local [Ca²⁺]_{cyt} transient in this simulation corresponds to a Ca²⁺ spark, characterized by Cheng and Lederer as having an approximate amplitude of 5 μM and a duration of 35 milliseconds in a space of 30 nm [55]. Under the current conditions, the duration of the spark is three times longer and a space of 90-100 nm would be expected. The activation of BK channels by spark coming from the ER has been described in smooth muscle [17] but not in neuronal cell bodies. The microdomains of Ca²⁺ consist of very small spaces (nm) between structures (e.g., voltage-gated Ca²⁺ channels and BK channels or IP₃-Rs), thus involving very local increases in Ca²⁺ [57]. Microdomains between BK channels and IP₃-Rs have been found in different cells, including neurons, with distances of 100 nm or less [23] [59].
Figure 3 presents four simulations with the following values of Ca$^{2+}$ in the endoplasmic reticulum pool: 100, 300, 600, and 700 μM. As a consequence, there is an increase in amplitude (5, 7, 10, and 11 μM) and duration (96, 235, 389, and 431 ms), respectively, of [Ca$^{2+}$]$_{cyt}$.

Where $V_3$ is the IP$_3$-sensitive Ca$^{2+}$ efflux out of the ER through its membrane and into the cytosol, $V_M$ denotes the maximum rate of such Ca$^{2+}$ release, and $R$[Ca$^{2+}$] depicts the fraction of the calcium channels in the open state, which depends on the [Ca$^{2+}$]$_{cyt}$ concentration. The function $R$ has been described by the same sort of mathematical formulations as for the opening of voltage-gated channels, in most cases either by Markov kinetic schemes or by Hodgkin-Huxley-style gating particles. The Goldbeter model uses a simpler approach with a suitable Hill coefficient, $n_{\text{Hill}}$ (Equation 17) \cite{32}.

**Interaction of IP$_3$-R/[Ca$^{2+}$]$_{cyt}$/BK channels**

The opening of BK channels is governed by membrane depolarization and a rise in the concentration of [Ca$^{2+}$]$_{cyt}$. Figure 4, shows the effect of a 5 μM increment in [Ca$^{2+}$]$_{cyt}$ on the action potential train in the presence of the BK channel. Each simulation has 100 μM of [Ca$^{2+}$]$_{ER}$, with all variables set at the previously described initial values. A range was established for the BK channel conductance values, generally considered as 0.1 μS/cm² in hippocampal neurons and 1.2 μS/cm² in sympathetic ganglion cells, in agreement with the values proposed by Traub and Miles and by Koch and Segev, respectively \cite{46} \cite{60}. Four simulations were carried out with a BK conductance of 0.1, 0.6, 1.2, and 1.5 μS/cm², affording the following results (respectively): no effect on the PA train (1500 ms), braking of the PA train at 1050 ms, fast braking at 83 ms, and braking immediately after the first action potential (values in green boxes in Figure 4 A, B, C, and D). Hence, there was a conductance-dependent effect of the [Ca$^{2+}$]$_{cyt}$: the higher the conductance, the greater the effect of the [Ca$^{2+}$]$_{cyt}$ concentration.

The dependence of the BK channel on the voltage is of an allosteric type. It has been proposed that the increase in [Ca$^{2+}$]$_{cyt}$ could occur almost simultaneously with a rise in voltage, as long as the calcium source is close, as in the case of the BK/VGCC complex \cite{60}. In the present study, a BK/IP$_3$-R complex is assumed. Each stimulus
\[ E = V + \gamma - \zeta \]

\[ g = g_{\text{BK}} - V^2 + \zeta \]

\[ \frac{k}{n_c} = \frac{k}{n_c} \] (\( k \) represents single-channel conductance (200 to 300 pS), and \( P_o \) is the open state probability value (0 to 1).

The activation of BK channels by a rise in \([\text{Ca}^{2+}]_{\text{cyt}}\) results in a greater open state probability and an enhanced K+ efflux. This is the molecular kinetics of channel opening induced by \[\text{Ca}^{2+}\] and voltage. The \[\text{Ca}^{2+}\] concentration continues to be optimal for a greater channel opening until the saturation point is reached. Consequently, the conductance for each cell depends on the number of channels, and the unit conductance of each one. The results of the simulation indicate that the ease of response of the neuron to the concentration of \[\text{Ca}^{2+}\] depends on the number of BK channels present in its membrane. The higher the conductance, the higher the response to \[\text{Ca}^{2+}\] and vice versa.

Subsequently, an evaluation was made of enhancing the rate of the \(\text{IP}_3\)-sensitive release of \([\text{Ca}^{2+}]_{\text{ER}}\) to the cytosol. The parameters of the neuron were fixed to the default values and the BK conductance set at 0.6 \(\mu\text{S}/\text{cm}^2\) (an intermediate value). By increasing the value of \(V_{\text{M3}}\) from 500 to 600, a higher level of \([\text{Ca}^{2+}]_{\text{cyt}}\) is obtained. An abrupt release of \[\text{Ca}^{2+}\] from the ER boosts the \([\text{Ca}^{2+}]_{\text{cyt}}\) from 5 to 6 \(\mu\text{M}\) (red lines). In the previous simulation (blue lines), corresponding to the effect of \([\text{Ca}^{2+}]_{\text{cyt}}\) at 5 \(\mu\text{M}\), the action potential train was found to brake at 721 ms. When the concentration of \([\text{Ca}^{2+}]_{\text{cyt}}\) is increased, calcium acts on the BK channel, stopping the action potential train after the second action potential (Figure 5). It is observed that at greater CICR pumping rates, the level of \([\text{Ca}^{2+}]_{\text{cyt}}\) rises by a calcium-induced calcium release process and/or by the direct action of \(\text{IP}_3\) on the calcium-\(\text{IP}_3\)-R channel (by Schetter and Smolen in \[59\]).

An increment in \(V_{\text{M3}}\) to 700 \(\mu\text{M/s}\) generates a rise in \([\text{Ca}^{2+}]_{\text{cyt}}\) to 7 \(\mu\text{M}\). Under these conditions, the neuron stops firing immediately after the first action potential (Figure 6). Although the model of Goldbeter contemplates the action of \(\text{IP}_3\) on \(\text{IP}_3\)-R and offers a good

\[ g_{\text{BK}}(V, [\text{Ca}^{2+}]_{\text{cyt}}) = n_c g_{o}(V, [\text{Ca}^{2+}]_{\text{cyt}}) \] (19)
approximation of the experimental data, it does not provide a dose-response relationship. It has been documented in the literature that the higher the level of IP$_3$, the greater the release of Ca$^{2+}$ from the ER [62]. Taking the limitations of the Goldbeter model into account, this simulation reasonably resembles the IP$_3$-sensitive Ca$^{2+}$ release from the [Ca$^{2+}$]$_{ER}$ pool.

According to the results, Ca$^{2+}$ released from the ER to the cytosol can effectively gate BK channels, and the main effect is to stop the spike train (Figure 6). These findings are consistent with reported experimental data. The Ca$^{2+}$ released from ER stores, specifically through IP$_3$Rs but not RyRs, produces pauses in the firing of spiny projection neurons due to the activation of BK and SK channels [63]. The author proposes two Ca$^{2+}$ signaling pathways:

1. action potential → VGCC → RyR → BK & SK → sAHP (slow afterhyperpolarization); and

2. mGluR/mACHR (metabotropic glutamate receptors / muscarinic acetylcholine receptors) → IP$_3$ → IP$_3$R → BK & SK → firing pause.

In the current contribution, a theoretical evaluation was made of the level of [Ca$^{2+}$]$_{cyt}$, mainly considering the interaction of IP$_3$-Rs with BK channels. The find-
The effect of accelerating the action of the ATPase pump (SERCA) was explored under conditions of high Ca\textsuperscript{2+} efflux (the previous simulation). An increase in the $K_2$ parameter (the threshold constant for pumping) from 1 to 2 μM decreases the concentration of [Ca\textsuperscript{2+}]\textsubscript{cyt} from 7 to 3.4 μM. As can be appreciated, the action potential train is reversed and the neuron fires continuously during the simulated time (Figure 7).

Under physiological conditions, the PMCA pump and the SERCA pump are responsible for avoiding an excess concentration of [Ca\textsuperscript{2+}]\textsubscript{cyt}, which could be toxic to the cell \cite{22} \cite{60}. Such an excess concentration of Ca\textsuperscript{2+} would inhibit IP\textsubscript{3}-R \cite{22}, Calcium oscillations have been demonstrated to move like waves with an amplitude in the order of nM \cite{21} \cite{36} and a range dependent on their diffusion and binding with chelating molecules \cite{18}. These oscillations have been proposed as signals for various cellular processes, including synaptic plasticity, regulation of neurotransmission, cell differentiation, apoptosis, embryonic development, and secretion \cite{21}. The abrupt influx of Ca\textsuperscript{2+} into the cell by voltage-gated calcium channels in the plasma membrane activates BK channels and triggers a change in action potential frequency \cite{18}. The results of the present simulation show how an abrupt, focused increase in [Ca\textsuperscript{2+}]\textsubscript{cyt} from the endoplasmic reticulum store, very similar to those described as “spark”, favors the activation of BK channels and produces a pause in the action potential train. This would imply that, under certain conditions, a mechanism capable of stopping neuronal signaling is activated.

**CONCLUSIONS**

A theoretical study of the IP\textsubscript{3}-R/BK channel interaction was carried out by means of a simulator. Consequently, it was possible to modify the parameters to regulate the concentration of Ca\textsuperscript{2+} in the cytosol in order to monitor the effects. The variables considered are related to the flow of Ca\textsuperscript{2+} from cytosol: out of the cytosol to the ER (the SERCA pump) and to extra-
cellular space (the PMCA pump), as well as into the cytosol from the ER (through the IP₃-Rs) and from extracellular space (the VGCCs). Additionally, the conductance of the BK channel and the parameters of the Goldbeter model are included.

Some insights were provided into the general character of the IP₃-R/BK channel interaction. (1) The \([\text{Ca}^{2+}]_{\text{cyt}}\) concentration required to activate BK channels varies in accordance with the conductance of the channel. With a greater conductance, the concentration of \(\text{Ca}^{2+}\) necessary for activation is lower. (2) The BK channel is activated by an abrupt release of \(\text{Ca}^{2+}\) from the ER. This increase in \(\text{Ca}^{2+}\) is local and resembles spark. (3) The amplitude and duration of the abrupt efflux of \(\text{Ca}^{2+}\) from the ER depends on the difference in the concentration between the free \([\text{Ca}^{2+}]_{\text{cyt}}\) and the free \([\text{Ca}^{2+}]_{\text{ER}}\). The higher the concentration of the latter, the greater the amplitude and duration of the efflux. (4) A rise in the concentration of \([\text{Ca}^{2+}]_{\text{cyt}}\) increases the concentration of \([\text{Ca}^{2+}]_{\text{cyt}}\) and favors the activation of BK channels. (5) An acceleration of the SERCA and/or PMCA pumps decreases the concentration of \([\text{Ca}^{2+}]_{\text{cyt}}\), thus reducing or avoiding the activation of BK channels. (6) A rise in \([\text{Ca}^{2+}]_{\text{cyt}}\) activates the BK channels, leading to an immediate pause or stop of the spike train. This function will allow the neuron to generate a firing pattern in bursts and under certain conditions be a possible mechanism for resetting or preventing sustained abnormal PA activities (7). The colocalization of the BK and IP₃-R channels in a microdomain is a necessary condition for the manifestation of the following pathway in a neuron: \(\text{IP}_3 \rightarrow \text{IP}_3\cdot\text{R} \rightarrow \text{Ca}^{2+} \rightarrow \text{BK channels} \rightarrow \text{pause (stop)}\). This sequence is the regulatory mechanism (8) For neurons with a low \(g_{\text{BK}}\), a high \([\text{Ca}^{2+}]_{\text{cyt}}\) concentration, and a greater difference between this parameter and free \([\text{Ca}^{2+}]_{\text{ER}}\) is necessary to activate BK channels. (9) In case of having an elevated level of \(g_{\text{BK}}\) conductance, neurons activate the BK channels more easily. The aforementioned theoretical results help to explain the experimental data of Clements et al. [63] and are in accordance with the proposal made by these authors about the existence of \(\text{Ca}^{2+}\) signaling pathways that mediate BK channel activation according to the level of \([\text{Ca}^{2+}]_{\text{ER}}\).

**AUTHOR CONTRIBUTIONS**

M.E.P.B. consultant in physiology and in charge of the simulated experiments. M.R.M. was responsible for programming the design of interfaces and figures. J.Q.P. supervised the union of the models and the bibliographic review. M.F.P.E. oversaw the programming and compilation of the simulators. A.R.L. consulting in neuroscience and programming, in charge of mathematical models and their numerical solution. All authors participated in the structural analysis, review, and correction of the work.

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