Physiological Role of Kv1.3 Channel in T Lymphocyte Cell Investigated Quantitatively by Kinetic Modeling

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Abstract

Kv1.3 channel is a delayed rectifier channel abundant in human T lymphocytes. Chronic inflammatory and autoimmune disorders lead to the over-expression of Kv1.3 in T cells. To quantitatively study the regulatory mechanism and physiological function of Kv1.3 in T cells, it is necessary to have a precise kinetic model of Kv1.3. In this study, we firstly established a kinetic model capable to precisely replicate all the kinetic features for Kv1.3 channels, and then constructed a T-cell model composed of ion channels including Ca2+-release activated calcium (CRAC) channel, intermediate K+ (IK) channel, TASK channel and Kv1.3 channel for quantitatively simulating the changes in membrane potentials and local Ca2+ signaling messengers during activation of T cells. Based on the experimental data from current-clamp recordings, we successfully demonstrated that Kv1.3 dominated the membrane potential of T cells to manipulate the Ca2+ influx via CRAC channel. Our results revealed that the deficient expression of Kv1.3 channel would cause the less Ca2+ signal, leading to the less efficiency in secretion. This was the first successful attempt to simulate membrane potential in non-excitable cells, which laid a solid basis for quantitatively studying the regulatory mechanism and physiological role of channels in non-excitable cells.

Introduction

Kv1.3 and IK (KCa3.1) are two kinds of potassium channels in T cells. Kv1.3 channels are activated upon the depolarization of the membrane potential, while IK channels are activated by the Calcium ion [1–3]. There are three types of T cells, Naive T cell, TeM and TemT cells [3]. These quiescent cells exhibit a similar K+ channel expression pattern with ∼300 Kv1.3 and ∼10 IK channels per cell. However, Kv1.3 channels are up regulated to ∼1500 in the activated TemT effectors and IK channels are up regulated to ∼500 in the activated TemT effectors [3]. Such high expression of Kv1.3 channels has been reported associated with many chronic inflammatory and autoimmune disorders such as multiple sclerosis (MS), type 1 diabetes mellitus (T1DM) and rheumatoid arthritis (RA) [4–7], and therefore Kv1.3 channel served as a potential therapeutic target for treatment of these diseases, which was indicated by the blockers of chemical messengers during activation of T cells. Based on the experimental data from current-clamp recordings, we successfully demonstrated that Kv1.3 dominated the membrane potential of T cells to manipulate the Ca2+ influx via CRAC channel. Our results revealed that the deficient expression of Kv1.3 channel would cause the less Ca2+ signal, leading to the less efficiency in secretion. This was the first successful attempt to simulate membrane potential in non-excitable cells, which laid a solid basis for quantitatively studying the regulatory mechanism and physiological role of channels in non-excitable cells.

[17]. Those properties enable us to identify the Kv1.3 from various lymphocyte K+ currents.

Besides Kv1.3, five main types of ion channels have been identified at the molecular level in T cells. They are Ca2+-release activated calcium (CRAC) channel, intermediate K+ (IK) channel, TASK channel (a two-pore domain potassium (K2P) channel), TRPM7 channel and Osmo-activated Cl− (Clswell) channel [3]. As a major calcium source in T lymphocyte cells, the CRAC channel, formed by the STIM1 and Orai1 subunits, leads a Ca2+ influx while depleting the endoplasmic reticulum (ER) Ca2+ store [18–20]. Since the intracellular Ca2+ can modulate various important physiological functions such as potassium channel gene expression and secretion, the Ca2+ influx via CRAC channels may form a positive feedback to induce the larger Ca2+ signal [3,18,21].

It is well-known that a typical resting effector memory T (TeM) cell contains ∼300 functional Kv1.3 channels and ∼10 functional IK channels on the surface membrane [2,3], which can prevent membrane potential from excessively larger depolarization [19,22–25]. During T cell activation, they are the major contributor to maintain the membrane potential that promotes Ca2+ influx. A negative membrane potential potential enhances Ca2+ entry by optimizing the electrochemical driving force for Ca2+ movement through CRAC channels [26–29], and inhibit the Kv1.3 and IK potassium channels by their specific inhibitors will significantly reduce the calcium signaling [8,9]. However, a clear pattern in quantitative description on the functional role that
Kv1.3 channel plays in regulating the membrane potential and the intracellular local and global Ca$^{2+}$ signaling remains wrapped.

Kinetic modeling provides a good way for studying and predicting the kinetic behavior of ion channels and their function in cells. Several kinetic models of Kv1.3 channel have been reported previously [12–14]. They did excellent works focused on the individual activation, inactivation or recovery characteristics.

The accuracy of the cell model is based on the comprehensiveness of the ion channel models it constitutes. In this study, we establish a novel Kv1.3 model capable to precisely describe the whole kinetic behavior of Kv1.3, using a software CeL [29]. Based on the Hodgkin-Huxley theory, a model cell with appropriate component of channels can be used to simulate the firing pattern of action potentials in excitable cells [30]. But the H-H model has never been used to simulate the membrane potentials in non-excitable cells. This is a first attempt to construct a T-cell model, composed of several model channels including Kv1.3, CRAC, IK and TASK channels, for mimicking the dynamic behavior of membrane potentials and intracellular Ca$^{2+}$ signaling in T cells. Although there is no action potential in T lymphocyte cells, it is still interesting to know the membrane potential performances after stimulation. Combined with the current-clamp experimental data with different amount of Kv1.3 channels blocked by ADWX-1 from T lymphocyte cells, we do quantitatively mimic all the changes in membrane potential and the corresponding Ca$^{2+}$ signal in the non-excitable model T-cell. Overall, a simulation framework has been provided for further studying the regulatory mechanism of other channels in T lymphocyte cells, which lays a solid basis for both the immunological and ion-channel fields.

**Materials and Methods**

**Ethics Statement**

Peripheral venous blood was obtained from healthy volunteers, who provided their written informed consent to participate in this study. The consent procedure and our research were approved by the Ethics Committee of the College of Life Sciences in Wuhan University (Permit Number: ECCLS 20120076).

**Cell culture and Transfection**

Full length cDNA for Kv1.3 was subcloned into pcDNA3.1/Zeo (Invitrogen), HEK293 cells were cultured in modified Eagle’s medium (DMEM, Gibco) supplemented with 10% fetal bovine serum (FBS, Gibco) at 37°C in a humified incubator with 5% CO$_2$. The day before transfection, cells were transferred into a 24-well plate and transiently transfected using lipofectamine 2000 (Invitrogen). HEK293 cells were cultured in modified Eagle’s solution (ECS) containing 140 mM NaCl, 5 mM KCl, 1 mM MgCl$_2$, 2.5 mM CaCl$_2$, 10 mM Hepes (pH 7.4), 10 mM glucose, 2 mM HEPES (pH 7.4) titrated with NaOH. All the chemicals were attained from Sigma.

**Preparation of human T lymphocyte cells**

Peripheral venous blood was obtained from healthy volunteers. Mononuclear cells were isolated by Ficoll-Hypaque density gradient. T cells were separated from PBMCs by using FACS with PE conjugated CD3 antibody and maintained in RPMI 1640 medium supplemented with 1 mM L-glutamine and 10% fetal bovine serum (Gibco) in a humidified, 5% CO$_2$ incubator at 37°C. Recordings were carried out in 24 hours after T lymphocyte cells preparation.

**Solutions**

For whole-cell patch recordings, the pipette solution contained the following (in mM): 130 KCl, 2.5 MgCl$_2$, 10 HEPES, 1 EGTA, 2 K$_3$ATP (pH 7.4) titrated with KOH, bath solution contained the following (in mM): 145 NaCl, 5 KCl, 1 MgCl$_2$, 2 CaCl$_2$, 0.8 Hepes (pH 7.4) titrated with NaOH. All the chemicals were attained from Sigma.

**Intracellular Ca$^{2+}$ Measurements**

T lymphocyte cells were incubated in 100 µl of extracellular solution (ECS) containing 140 mM NaCl, 5 mM KCl, 1 mM MgCl$_2$, 1.8 mM CaCl$_2$, 10 mM Hepes (pH 7.4), 10 mM glucose, 2 µM fura-2/AM (Invitrogen), 2 mM Probenecid, 0.05% Pluronic F-127 and 0.1% bovine serum albumin at 37°C for 30 min. After the incubation, cells were placed in the platform for optical imaging. The imaging of intracellular Ca$^{2+}$ was performed on Olympus-IX70 microscope system with a polychromatic light source (T.I.L.L. Photonics GmbH, Graefeling, Germany). The excitation wavelengths of fura-2 fluorescence were 340 nm and 380 nm, coming from the bottom of plate at 1 Hz. All of the experiments were performed at room temperature (20–24°C).

**Data analysis**

Recording data were analyzed with IGOR (WaveMetrics, Lake Oswego, OR), Clampfit (Molecular Devices, Inc.) and Sigmaplot software (SPSS, Inc.). Unless stated otherwise, the data are presented as mean ± S.D. The conductance of Kv1.3 channels was calculated from $G = I_{peak}/(V-E_{Kv1.3})$, where $I_{peak}$ is the peak current, $E_{Kv1.3}$ is the reversal potential of Kv1.3. The conductance-voltage (G-V) curves of activation were fitted by the Boltzmann equation:

$$G/G_{max} = (1 + \exp(-(V-V_{50})/k))^{-1},$$

(1)

Where $G_{max}$ is the maximum conductance, $V_{50}$ is the half maximal conductance voltage and $k$ is a slope factor. The steady-state inactivation curve was fitted to the Boltzmann equation:

$$I_{peak} = I_{max}/(1 + \exp((V-V_{50})/k))$$

(2)

Where $I_{max}$ is the maximal peak current, $V_{50}$ is the half maximal availability voltage and the $k$ is a slope factor.

**Mathematical modeling and simulation**

T lymphocyte model cell has been described as the following equations [31]:

$$C_m * dV/dt = I_{inj} + I_{IK} + I_{CRAC} + I_{Kv1.3} + I_{TASK}$$

(3)

where $V$ is the voltage in mV, $C_m$ is the capacity of the cell membrane in pF, $t$ is the time in ms, $I_{inj}$ is the injection current in pA, $I_{IK}$ is the leak current in pA, $I_{CRAC}$ is the CRAC current in pA, $I_{Kv1.3}$ is the Kv1.3 current in pA and $I_{TASK}$ is the IK current in pA.
where the single-channel conductance \( g_{IK} \) and quantity of various buffers and the calcium concentration high enough (about 500 nM) after CRAC channel opening, which could be measured in T cells [41,42]. Their voltage dependence, rates of opening and closing, single-channel conductance, inactivation properties, and blockade by different compounds including tetraethylammonium (TEA) and charybdotoxin (CTX) were indistinguishable from native Kv1.3 channels. So we recorded Kv1.3 dynamics from transfected HEK293 cells to simplify the modeling.

To complete the kinetic model of Kv1.3, experiments were performed on Kv1.3-transfected HEK293 cells to acquire the activation (Fig. 1B), steady-state inactivation (Fig. 1C) and deactivation (Fig. 1D), respectively. In Fig. 1B, Kv1.3 currents (black) exhibited a fast activation and then a slow inactivation, evoked by 1 s depolarizing voltage steps ranging from −70 to +50 mV in 15 mV increments from a 90 s holding potential of −90 mV to remove the possible inactivation. The forward rates of the process \(\text{C} \rightarrow \text{O} \rightarrow \text{I} \) could be determined by fitting it to the activation currents. Here the red lines conferred a perfect fit (Fig. 1B). The voltage dependence of steady-state inactivation currents. Here the red lines conferred a perfect fit.
Similarly, the backward rate of \( C \rightleftharpoons O \) could be determined by fitting it to deactivation currents. All the fits (red) were fully overlapping to the data (black) (Fig. 1D).

The normalized activation (G-V) curves (black) of Kv1.3 had an averaged value of \( V_{50} = -228.4 \pm 1.6 \) mV, and the normalized steady-state inactivation (availability) curve tested at 50 mV had a \( V_{50} = -43.5 \pm 3.7 \) mV (Fig. 1E left), consistent with previous work on Kv1.3 [37,46,47]. In Fig. 1E, both the G-V and steady-state curves from simulations (red) were closely coincides with that of data (black). Additionally, the time constants of activation (\( \tau_a \)) (Fig. 1F bottom), inactivation (\( \tau_i \)) (Fig. 1F top), and deactivation (\( \tau_d \)) (Fig. 1G) were also consistent to the experimental data.

Based on the perfect fitness to the activation, inactivation, deactivation and steady-state inactivation data, all the parameters in Kv1.3 model were basically determined. Then we further examined whether this model matched the data of recovery from inactivation by subtly readjusting the parameters in the Kv1.3 model. Since the time course of recovery from inactivation is mono-exponential, the sequential model with only one inactivation state is good enough for the Kv1.3 kinetics. This model conferred by and large a rough fit to both the currents recovering at \(-45\) and \(-90\) mV. Fig. 2C displayed a set of fractional recovery curves arising from a two-pulse protocol (\( P_1 = 1 \) s, \( P_2 = 150 \) ms, \( V = -100, -90, -90 \) and \(-45 \) mV). The time constants of recovery were \( 8.6 \) s at \(-45 \) mV, \( 15.6 \) s at \(-80 \) mV, \( 14.6 \) s at \(-90 \) mV, \( 14.6 \) s at \(-100 \) mV, approximately consistent to the experimental data (Fig. 2D). Interestingly, this result indicated that the recovery of Kv1.3 was weakly voltage-dependent under physiological condition [14]. So far, a sequential kinetic model, well illuminating the whole kinetic behavior of Kv1.3 channels, was successfully established. All the parameters in model were listed in Table 1.

Scorpion toxin ADWX-1 is a pore blocker of Kv1.3 channel without affecting its kinetics

As we reported previously, the engineered scorpion toxin ADWX-1 is a potent specific inhibitor of Kv1.3. It has the highest affinity in \( \sim pM \) to Kv1.3 channels [17]. In Fig. 3A-B, 30 pM ADWX-1 blocked the Kv1.3 currents without changing the G-V curve (Fig. 3C) and the time constants of activation and inactivation (Fig. 3D). This means that the inhibition by ADWX-1 only reduces the number of open channels without affecting the channel kinetics. In other words, we can explore the
Kv1.3 role in T cell by simply changing the number of Kv1.3 channels in applying with different ADWX-1 concentrations.

Inhibition and characterization of Kv1.3 channels in T cells

Based on the sequential kinetic model of Kv1.3 channels, their inhibition features were further investigated in T cells. We first examined the voltage-activated currents in T cells in the absence and presence of 30 and 300 pM ADWX-1. Our results demonstrated that the slow inactivated currents were sensitive to ADWX-1 (Fig. 4A). And after applying 300 pM ADWX-1 to T cell, there is little outward current left at 50 mV, indicating that Kv1.3 current was the major prominent of outward current in this case. In current-clamp experiments, we also found that the membrane potentials of T cells rapidly went to depolarization direction by 15 pA current injection while Kv1.3 channel were inhibited by ADWX-1 (Fig. 4B, green and blue), in contrast, the membrane potential changed much slowly without ADWX-1 (Fig. 4B, black).

To better understand the regulatory mechanism of Kv1.3 channels in T cells, we constructed a model T-cell, composed of

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**Table 1. Parameters of Kv1.3 model.**

| Parameter | Values          |
|-----------|-----------------|
| a         | 0.448 (ms\(^{-1}\)) |
| b         | 0.043 (ms\(^{-1}\)) |
| c         | 0.003 (ms\(^{-1}\)) |
| d         | 0.00008 (ms\(^{-1}\)) |
| A         | 280.035 (ms\(^{-1}\)) |
| B         | 1.648 (ms\(^{-1}\)) |
| m         | 27.530 (mV) |
| n         | 17.528 (mV) |
| p         | 174.961 (mV) |
| q         | 1016.330 (mV) |

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Figure 2. Comparison of the recovery of Kv1.3 channels between the experimental data and simulations. (A–B) The representative currents of recovery from inactivation were obtained in whole-cell patch mode at −90 mV and −45 mV. Two-pulse protocol: from a holding potential of −90 mV, the first pulse 1 (P1) was applied for 1 s at +50 mV, and then the second +50-mV pulse (P2) was applied for 150 ms after a variable interpulse interval at −90 mV in (A) and −45 mV in (B). The detailed recovery currents are shown in the box. The voltage protocol is placed at the bottom. Red lines are fits and black ones are data. The channel numbers for simulations are 3788 for −90 mV and 2740 for −45 mV. (C) Comparison of the time courses of recovery between the experimental data (black) and the simulations (red) at −100 mV, −90 mV, −80 mV and −45 mV. (D) Comparison of time constants of recovery between the experimental data (black) and the simulations (red). The time constants of recovery (mean ± SEM) are 8.6 ± 2.7 s (n = 5) at −45 mV, 15.6 ± 1.6 s (n = 3) at −80 mV, 14.6 ± 2.4 s (n = 7) at −90 mV, 14.6 ± 4.7 s (n = 3) at −100 mV, respectively.

doi:10.1371/journal.pone.0089975.g002
the Kv1.3 channel, Ca\(^{2+}\)-release activated calcium (CRAC) channel, intermediate K\(^{+}\) (IK or \(K_{Ca}3.1\)) channel and leak K\(^{+}\) (TASK) channel [3], to simulate the membrane potentials in a T cell, based on the Eq. (3–5) and the open probability \(P_o\) of each channels (See the Methods and Materials). For more precise, we calculated the time- and voltage-dependent total conductance of Kv1.3 via the open probability \(P_o\) of Kv1.3 kinetic model rather than via the formulized term \(m^3 h\) in classic Hodgkin-Huxley model (See Eq. (4)). Essentially, they are the same. According to this T-cell model, our simulations revealed that the total conductance of Kv1.3 was 4.5 nS (\(N_{Kv1.3} = 300\)) for control, 2.0 nS (\(N_{Kv1.3} = 133\)) for 30 nM ADWX-1 and 0.4 nS (\(N_{Kv1.3} = 27\)) for 300 pM ADWX-1 (Fig. 4B). The corresponding membrane potential depolarization property in these situations depended on the number of Kv1.3 channels, indicating that Kv1.3 played a dominative role in maintaining the membrane potential of T cells. In order to further validate the accuracy of the Kv1.3 channel model and T cell model, we used one membrane potential trace recorded from a T cell as a voltage command to stimulate the other T cells, then we obtained a hump-shaped Kv1.3 current, which could be well reproduced by the Kv1.3 kinetic model, by using the same voltage command (Fig. 4C). This showed that the Kv1.3 current started to be activated at about −40 mV and then reached to the maximum at −40 mV, and finally decreased to the minimum at more positive voltages due to inactivation. In this simulation, we found that the number of Kv1.3 was about 330, consistent to the previous report [3]. Note that here we subtracted the background current by applying with 1 nM ADWX-1 to get the pure Kv1.3 current.

The membrane potential of T cells is governed by Kv1.3 channels

As the T cell is a secretory cell [48], it is important to know the intracellular local and global Ca\(^{2+}\) signaling. Preferably, it is to know the local Ca\(^{2+}\) signaling inside T cells. Since the I-V curve of CRAC channel is substantially linear and the membrane potential is the only driving force to promote the Ca\(^{2+}\) influx [26–28], we first calculated the membrane potentials in a model T-cell containing different amount of Kv1.3 channels. Fig. 4D illustrated that the changes in membrane potentials of T cells clearly depended on the total conductance (or number) of Kv1.3 channels. Here we chose several typical numbers of Kv1.3 channel in T cell. For example, the total conductance of 22.5 nS implied the number of Kv1.3 was 1500 as a single-channel conductance of Kv1.3 was 15 pS [36]. An activated T cell with 1500 Kv1.3 channels could be considered as an effector memory TEM cell [3].

Afterwards, we further calculated the corresponding local calcium signaling based on Eq. (5). The calculated Ca\(^{2+}\) signaling exhibited a Ca\(^{2+}\) peak at negative potentials, following by a lower Ca\(^{2+}\) plateau at positive potentials (Fig. 4E). The maximal local Ca\(^{2+}\) concentration around vesicles is up to over 20 μM, which would be sufficient to induce T-cell exocytosis [45]. Our results
revealed that the Ca$^{2+}$ signaling strongly depended on the numbers of Kv1.3 channel. The larger amount of Kv1.3 had the higher Ca$^{2+}$ plateau. In other words, the less Kv1.3, the less [Ca$^{2+}$]$_i$ for secretion, suggesting that a T cell lacking of Kv1.3 might lose function. Recently, it was found that the Kv1.3 knockout mice produced lower incidence and severity of T cell-mediated autoimmune encephalomyelitis [49], which was in line with our proposed model that the less Kv1.3 channels would inhibit the calcium entry into T cells and likely further affected the cytokine production. The Kv1.3, IK and CRAC currents in the cases of Figure 4 D–4E were shown in Figure 1. Note that the inactivation property of Kv1.3 channel caused a pseudomorph paradox of the larger conductance conferring the smaller currents, which was actually derived from the smaller open probability due to inactivation of Kv1.3 channel. Nevertheless, Kv1.3 currents were still much larger than others, indicating that neither CRAC nor IK could significantly affect membrane potentials as Kv1.3 could. This also suggested that the intracellular Ca$^{2+}$ was dominated by Kv1.3 channels.

The CRAC channel was triggered by IP3-induced depletion of the ER Ca$^{2+}$ store [18–20]. In Fig. 4F, however, we found that the 60% lower osmolarity solution induced a global augment of intracellular Ca$^{2+}$ in T cells, consistent to the report by Ross and Cahalan [40]. The low osmolarity solution activated a swelling-activated Cl$^-$ (Cl$_{swell}$) channel by swelling T cell, leading depolarization of T cell [3,50,51]. The time course of the global Ca$^{2+}$ concentrations showed a Ca$^{2+}$ peak at beginning, following by the higher Ca$^{2+}$ plateau, comparable with that as predicted by the model T cell (Fig. 4E). Obviously, the slow effect of the regulatory volume decrease (RVD) could not be the major reason for the Ca$^{2+}$ decay in seconds, because it occurred in minutes. Additionally, it was not clear whether the low osmolarity can directly...
trigger the opening of the CRAC channel or stretch-activated cation channel with a reversal potential $V_{rev} \approx 0$ mV. For simulation, there was actually no essential difference between the open of CRAC and stretch-activated cation channels at about $-40$ mV ($<0$ mV) in applying with the lower osmolarity solution [3], because the calcium influx derived from either the CRAC or cation channel was governed by Kv1.3 channels.

Discussion

The effector memory TEM cell, composed of Kv1.3highKCa3.1low has been confirmed for several human autoimmune diseases, such as multiple sclerosis (MS), type 1 diabetes mellitus (T1DM) and rheumatoid arthritis (RA) [5–7]. The Kv1.3 voltage-gated potassium channel regulates membrane potential and calcium signaling in TEM cells that are key mediators of autoimmune diseases, a thoroughly studying the physiological role of Kv1.3 channel plays in T cell not only helps to understand the working mechanism of T cell, but also provide a crucial clue for treatment of diseases.

In this study, we systematically studied the basic characteristics of Kv1.3 channel and constructed a sequential kinetic model capable to precisely replicate all the kinetics of this channel. Moreover, we examined the Kv1.3 currents in T lymphocyte cell by a specific inhibitor ADWX-1 toxin, consistent to that of expressed in HEK293. To explore the physiological role of Kv1.3 channel, we further built a T model cell, which was composed of four basic channels prominently in T lymphocyte cell. Finally, we demonstrated that the Kv1.3 channels could dominate the membrane potentials of T lymphocyte cell, and that the CRAC channels manipulated the intracellular Ca2+ signaling along with changes of membrane potentials.

Using the model T-cell composed of four channels, we have examined the mechanism of Kv1.3 channel regulating T lymphocyte cells. Actually, a couple of channels, i.e., TRPM7, Osmo-activated Ca2+(Clswell), and TRPC3/6 were excluded from this T-cell model. The TRPM7 (or a Mg2+-inhibitor-Ca2+ (MIC) permeable channel) is stretch- and swelling-activated cation channel, which can be activated by PIP2 and inhibited by Mg2+ [52,53]. The Clswell channel conducts an outwardly rectifying chloride current (or volume-regulated anion current), leading an anion and osmolyte efflux that is activated by cell swelling [54]. In our experiments, TRPM7 current was blocked by intracellular 2.5 mM Mg2+. Besides CRAC channel, TRPC3/6 channels provide another Ca2+ entry pathway, but it seems that these two channels are little expressed in T cells, the amplitude of TRPC3 inward current is only around 2 pA/pF (or 1–3 pA/cell) at −50 mV [55], which could be neglected in our model, so the model T-cell composed of four basic channels is thus good enough for this work.

During the whole depolarization process of membrane potentials, CRAC contributes much less current than that of Kv1.3, due to the small total conductance of 0.09 nS compared with ~4.5 nS of Kv1.3. And the membrane potential was much less sensitive to the CRAC channel conductance in simulations, suggesting that Kv1.3 played the key role in dominating membrane potentials in T cells. While CRAC channel was the major Ca2+ source in T cells, which provides a continuous calcium influx to elevate the intracellular Ca2+ level for T-cell exocytosis. With TASK channels together, Kv1.3 channels also play a role in maintaining the rest potential.

In this study, we identified the physiological role of Kv1.3 channels, which further provided us an opportunity to better understand the activation mechanism of T cells. How can a CRAC channel in a T cell be activated following antigen stimulation? In Fig. 5, a cartoon shows that an activated T cell increases the surface expression of Kv1.3, to negative-shift the membrane potential to increase the intracellular Ca2+ via CRAC channels, and finally to start the IL-2 (interleukin 2) promoter production program [3]. This process is more like a positive feedback [3,18], possibly terminated by endocytosis due to internationalization of Kv1.3 channels. This will decrease the number of Kv1.3 on plasma membrane to lead T cell gradually return to its resting state again.

In this study, we provided a precise model of Kv1.3 and a novel non-excitatory model for quantitatively investigating the regulatory mechanism of T cell, which laid a solid basis for further studying other channel such as IK, TASK roles in T cell and the events of membrane potentials, calcium signaling and secretion in other non-excitatory cells such as TCM, B and sperm cells.

Author Contributions

Conceived and designed the experiments: JD YW RZ PH. Performed the experiments: RZ PH YL WW. Analyzed the data: RZ PH WW. Contributed reagents/materials/analysis tools: JF YL WW. Wrote the paper: JD YW RZ PH.
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