

Structure, Gating and Basic Functions of the Ca^{2+} -activated K Channel of Intermediate Conductance

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Abstract: Background: The KCa3.1 channel is the intermediate-conductance member of the Ca^{2+} -activated K channel superfamily. It is widely expressed in excitable and non-excitable cells, where it plays a major role in a number of cell functions. This paper aims at illustrating the main structural, biophysical and modulatory properties of the KCa3.1 channel, and providing an account of experimental data on its role in volume regulation and Ca^{2+} signals.

Methods: Research and online content related to the structure, structure/function relationship, and physiological role of the KCa3.1 channel are reviewed.

Results: Expressed in excitable and non-excitable cells, the KCa3.1 channel is voltage independent, its opening being exclusively gated by the binding of intracellular Ca^{2+} to calmodulin, a Ca^{2+} -binding protein constitutively associated with the C-terminus of each KCa3.1 channel α subunit. The KCa3.1 channel activates upon high affinity Ca^{2+} binding, and in highly coordinated fashion giving steep Hill functions and relatively low EC50 values (100-350 nM). This high Ca^{2+} sensitivity is physiologically modulated by closely associated kinases and phosphatases. The KCa3.1 channel is normally activated by global Ca^{2+} signals as resulting from Ca^{2+} released from intracellular stores, or by the refilling influx through store operated Ca^{2+} channels, but cases of strict functional coupling with Ca^{2+} -selective channels are also found. KCa3.1 channels are highly expressed in many types of cells, where they play major roles in cell migration and death. The control of these complex cellular processes is achieved by KCa3.1 channel regulation of the driving force for Ca^{2+} entry from the extracellular medium, and by mediating the K^+ efflux required for cell volume control.

Conclusion: Much work remains to be done to fully understand the structure/function relationship of the KCa3.1 channels. Hopefully, this effort will provide the basis for a beneficial modulation of channel activity under pathological conditions.

Keywords: KCa3.1, gating, calmodulin, NDPK-B, PKA, volume regulation, calcium influx.

1. INTRODUCTION

The intermediate conductance Ca^{2+} -activated K channel, KCa3.1 (also known as KCNN4, IKCa, SK4), belongs to the Ca^{2+} -activated K channel (KCa) family. Their members, originally classified according to single-channel conductance into large- (KCa1.1), intermediate- (KCa3.1), and small-conductance (KCa2.1-3) K channels, were later found to form two well defined and distantly related groups, based on genetic relationship and mechanisms of Ca^{2+} activation. One of these groups includes the KCa1.1 channel that displays large unitary conductance and is gated by the cooperative

action of membrane depolarization and $[\text{Ca}^{2+}]_i$, while the other group includes both the intermediate-conductance KCa3.1 and small-conductance KCa2.1-3 channels, all showing much smaller unitary conductance and gated solely by $[\text{Ca}^{2+}]_i$. Unlike the KCa1.1 channel, the members of this second group do not bind Ca^{2+} directly, but rather detect it *via* calmodulin (CAM), which is constitutively bound to their C-terminal region (see later). Binding of Ca^{2+} to CAM results in conformational changes that are responsible for channel gating.

The KCa3.1 channel was first cloned in 1997 from human pancreas [1]. Initial biochemical and electrophysiological analysis showed that KCa3.1 channels were diffusely expressed in many different cell types, but not in the brain [1, 2]. KCa3.1 channels were however highly expressed in established cell lines from brain tumors, as well as in brain

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tumors *in situ* [3], and in many other tumor types. Only much later the KCa3.1 channel was described in microglia and most recently in hippocampal neurons and the nodes of Ranvier of cerebellar Purkinje neurons [4-6].

The biophysical properties of the KCa3.1 channel have been largely investigated using various experimental approaches and cell models. Sample data from our laboratory obtained on cultured human glioblastoma cells, and consistent with most studies, show that unitary currents display moderate inward rectification, single-channel conductance of 25 pS, and high selectivity for K^+ vs. Na^+ (Fig. 1B). The channel is not gated by voltage (Fig. 1A and B, inset), but sensitive to $[\text{Ca}^{2+}]_i$; changes, with a P_o vs. $[\text{Ca}^{2+}]_i$ relationship well fitted by a Hill relationship with IC_{50} of 188 nM and Hill coefficient of about 3.2 (Fig. 1D). Notably, P_o reached a maximum value much smaller than unity (~ 0.44) at $[\text{Ca}^{2+}]_i$ greater than $\sim 1 \mu\text{M}$ [7].

Much investigation has also addressed the pharmacology of KCa3.1 channels. The peptidic toxins charybdotoxin (ChTx) and maurotoxin (MTx) are high-affinity inhibitors of KCa3.1 (IC_{50} in the nM range), although only MTx, showing no significant inhibition of the other KCa channels up to $1 \mu\text{M}$ [8], can be considered selective. Small synthetic molecules have also been developed, mostly on the clotrimazole template, the classical KCa3.1 blocker. The most widely used TRAM-34 inhibits KCa3.1 with an $\text{IC}_{50} \sim 20 \text{ nM}$, and displays high selectivity over the other KCa channels [9]. As for KCa3.1 activators, the benzimidazolone 1-EBIO activates the channel with an $\text{EC}_{50} \sim 30 \mu\text{M}$ [10, 11], while the related molecule DC-EBIO exhibits ten-fold higher potency. Two more potent activators are the oxime NS309 and the benzothiazole SKA-31, with $\text{EC}_{50} \sim 250 \text{ nM}$ [12, 13].

This review will focus on the KCa3.1 channel general structure, channel gating, its functional and expression

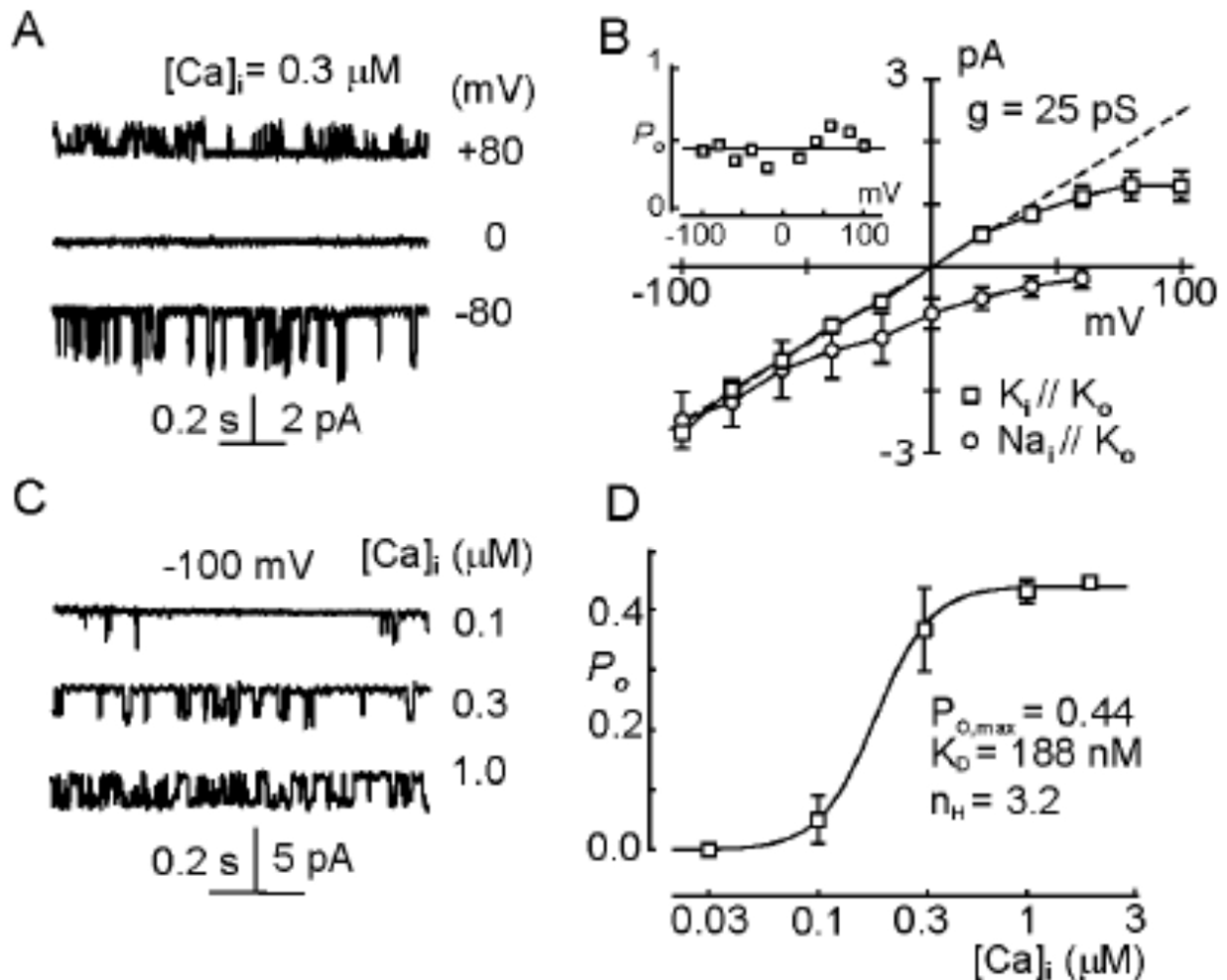


Fig. (1). Biophysical properties of KCa3.1 channel in human glioblastoma GL-15 cells. A) representative single-channel recordings at varying voltages in symmetrical 150 mM K^+ and $0.3 \mu\text{M}$ $[\text{Ca}^{2+}]_i$. B) current-voltage relationship constructed through a double Gaussian fit of the current amplitude histograms made from the unitary currents of the type shown in panel A, under symmetrical 150 mM K^+ (\square) and after substitution of internal K^+ with Na^+ (\circ). Dashed line, representing linear fit of the 3 control data points at the most negative voltages, gives a slope conductance (γ) of 25 pS for this channel. Inset: open probability (P_o) vs. voltage for the patch shown in A. C) inside-out single-channel recordings at -100 mV in symmetrical K^+ conditions at the indicated $[\text{Ca}^{2+}]_i$. D) KCa3.1 channel P_o vs. $[\text{Ca}^{2+}]_i$. The solid line represents the best fit of the experimental data with a Hill function (best-fit parameters are indicated) From [7].

modulation, and the basic cellular functions the channel is involved into.

2. STRUCTURE AND GATING OF THE KCa3.1 CHANNEL

2.1. General

The KCa3.1 channel is encoded by the gene *KCNN4*, which is localized in the q13.2 locus of human chromosome 19 [14]. The KCa3.1 channel was first described by Gardos in 1958, on noticing a link between the K efflux from erythrocytes and the intracellular Ca^{2+} level [15]. Four decades later, cloning and functional expression of the KCa3.1 channel have shown that the KCa3.1 is a tetrameric membrane protein, with each subunit organized in six transmembrane segments, S1–S6, with a pore motif between segment S5 and S6 (Fig. 2). The Ca^{2+} sensitivity is conferred to the KCa3.1 channel by CAM [16], which is constitutively bound (through its C-lobe) to a domain in the membrane-proximal region of the intracellular C terminus of the channel, thus serving as the channel's Ca^{2+} sensor. Binding of Ca^{2+} to CAM results in changes in the CAM conformation and subsequent opening of the channel.

2.2. Calmodulin and its Binding to the KCa3.1 Channel

CAM is a small, highly conserved protein of 148 amino acids (~17 kD), with two globular lobes (N- and C-lobe), each containing two pairs of helix-loop-helix (EF-hand) motifs for a total of four Ca^{2+} binding sites. The two lobes are connected by a long, highly flexible α helix that allows the protein to adopt different conformations and varying affinity

to target proteins upon Ca^{2+} binding. The four EF-hand Ca^{2+} -binding sites, numbered I to IV starting at the N-terminal part of the protein, display very different affinities. Ca^{2+} binding to CAM is best described by a sequential binding model with high cooperativity, whereby Ca^{2+} binding to the highest affinity site (site III) triggers conformational changes that increase Ca^{2+} binding affinity to the other sites [18, 19].

The first evidence for CAM representing the Ca^{2+} sensor of KCa3.1 channels was reported by [16]. Using yeast two hybrid system and deletion analysis they found that CAM interacts strongly and in a Ca^{2+} -independent manner with the first 62 aminoacids of the C-terminal tail of KCa3.1 (Fig. 2). Notably, C-terminal fragments devoid of the first 37 aminoacids could bind CAM only in presence of Ca^{2+} , anticipating the presence of Ca^{2+} -dependent and independent interactions (see below). Furthermore, the expression of a mutant CAM defective in Ca^{2+} sensing reduced KCa3.1 channel activity, suggesting that Ca^{2+} -CAM binding is necessary for the channel to open, and the functional effect of the coexpression of WT and mutated CAM in varying proportions was in accordance with a model where Ca^{2+} binding to all four CAM/KCa3.1 subunits is necessary to open the channel. Finally, it was observed that CAM antagonists that prevent CAM binding to its target protein are able to inhibit the KCa3.1 current, a result that suggests the reversibility of the CAM-KCa3.1 interaction [20].

2.3. Gating of the KCa3.1 Channel

The exact molecular mechanism underlying KCa3.1 channel opening in response to Ca^{2+} binding to the CAM/KCa3.1 channel complex has not been fully eluci-

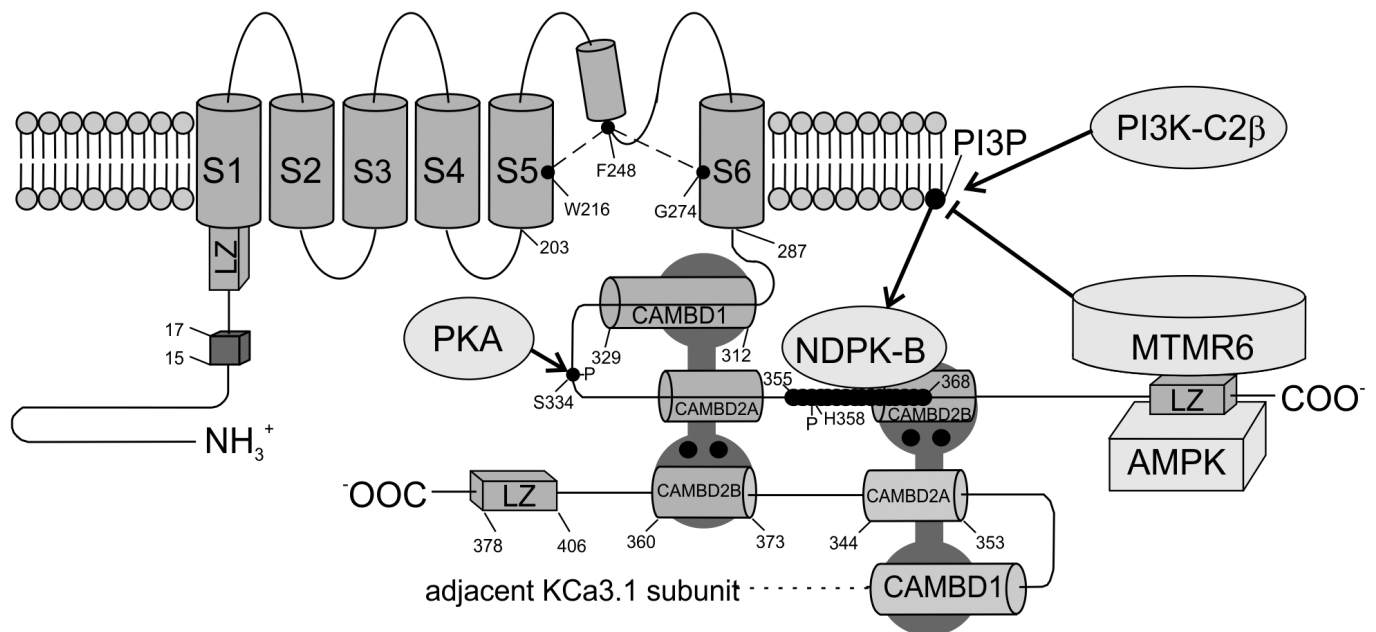


Fig. (2). Structure and membrane topology of the KCa3.1 channel. KCa3.1 is a tetrameric protein, with each subunit organized in six transmembrane segments plus a pore region between segments 5 and 6. The channel Ca^{2+} sensitivity is conferred by CAM, with the CAM C-lobe constitutively bound to the 312-329 segment of the channel C-terminal region (CAMBD1). The Ca^{2+} -dependent binding of the CAM to the channel with the CAM N-lobe involves the segments 360 to 373 (CAMBD2B) and 344 to 353 (CAMBD2A). The proteins and channel residues relevant to the modulation of the channel by PKA, AMPK, and PI3P are shown.

dated, mainly because lack of high resolution 3-D structures of KCa3.1 channel. The only structural information on KCa3.1 channel gating derives from the crystallization of CAM bound to the rat KCa2.2/CAM-binding domain (CAMBD) in the presence of Ca^{2+} [21]. It was observed that two adjacent C-terminal segments from two subunits of the same channel are connected, in a Ca^{2+} -dependent manner, by two CAM molecules to form an elongated dimer (Fig. 2). This dimerization of contiguous channel subunits would in turn lead to a rotation/translation of the associated S6 helices and the opening of the ion-conducting pore [21-24] (Fig. 3). Kinetic measurements of KCa2, where terbium was used as CAM ligand instead of Ca^{2+} , confirmed that ligand binding at the N-lobe low affinity sites of CAM is responsible for channel opening [25, 26].

A recent structural homology model based on the crystal structure of the KCa2.2/CAM complex has predicted also for KCa3.1 channels the presence of a Ca^{2+} -dependent two-CAM/two-subunit complex [27] (Fig. 2). The model suggests that the CAM C-lobe would bind in a Ca^{2+} -independent manner to a region of the C-terminal tail of the channel including residues 312-329 (CAMBD1, Fig. 2), while the N-lobe would bind, only in presence of high Ca^{2+} , the same subunit in a nearby segment (344-353, CAMBD2A) and an adjacent subunit in a more distal segment (360-373, CAMBD2B; cf. Fig. 2), leading to dimerization and channel opening.

As regard to the location of the activation gate, SCAM (substituted cysteine accessibility method) analysis of the KCa3.1 channel indicates that it is not located at the bundle crossing, but deeper in the channel pore, as cysteine modifying agents with dimensions wider than the hydrated K^+ ion may access residues deeper in the S6 segment also when the channel is closed [28, 29]. In accordance, mutagenesis experiments indicate that residue F248 of the putative inner pore helix of the KCa3.1 channel, located very close to the selectivity filter (Fig. 2), interacts with residue W216 of the

S5 helix, and this interaction stabilizes the closed conformation of the channel. However, F248 may also interact with residues close to the gating hinge G274 of the S6 helix, leading to a stabilization of the open state (Fig. 2; [30]). These data suggest that during channel gating the inner pore helix changes its position relative to the S5 helix, leading to a rearrangement of the channel selectivity filter and to a change in the channel permeation properties. Gating at the level of the selectivity filter would in addition explain the low maximal open probability (P_{max}) of KCa3.1 channels at saturating Ca^{2+} concentrations ([31, 32]; Fig. 1D). This occurrence would suggest a mechanism whereby the binding of Ca^{2+} to the CAM/KCa3.1 complex brings the channel to a closed/pre-open state from which the open state is reached in a Ca^{2+} independent way and with unfavorable energetics. In this regard, results obtained with mutant KCa3.1 channel in the S6 segment, or after chemically-induced modifications on the same segment, assign an important role to the S6 segment conformational state in determining P_{max} [28, 33].

3. MODULATION OF THE KCA3.1 CHANNEL FUNCTION AND EXPRESSION

3.1. Modulators of Gating

Several studies have shown that the KCa3.1 channel activity represents an integration point of multiple cell signaling pathways. KCa3.1 channel modulation by cAMP-activated protein kinase A (PKA) has been variously reported, with results showing differences in species, cell type and experimental approach [34-41]. An inhibitory effect of PKA on human and rat KCa3.1 channels was more recently shown to be due to channel phosphorylation at the PKA-specific phosphorylation site S334 (cf. Fig. 2), and consequent reduction of CAM binding affinity for the channel [42]. Since S334 is localized very close to the Ca^{2+} -independent CAM binding domain (CAMBDA), these data suggest a modulatory mechanism whereby phosphorylation

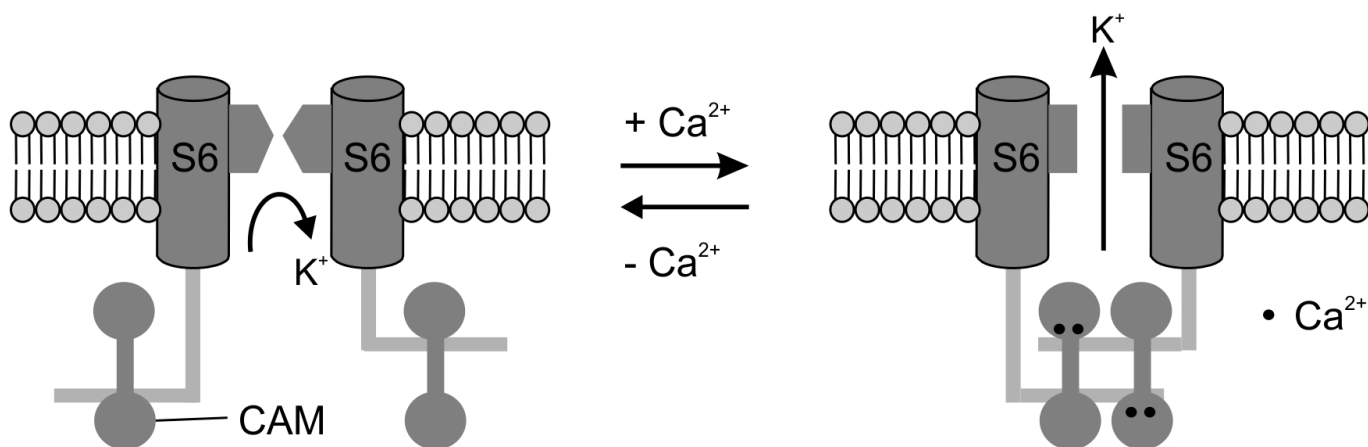


Fig. (3). Schematic illustrating the suggested Ca^{2+} -induced dimerization of the intracellular C-terminal segments leading to KCa3.1 channel opening, as deduced from studies on the SKCa channel. The crystal structure of the SKCa channel obtained in the presence of Ca^{2+} ions indicates that the Ca^{2+} /CAM/CAMBD complex forms a dimer structure in which two antiparallel C-terminal segments of two adjacent channel subunits are joined together by two CAMs.

of S334 leads to a conformational change promoting the unbinding of CAM from the channel, and the consequent reduction in channel activity.

5'AMP-activated protein kinase (AMPK) is a serine/threonine kinase ubiquitously expressed, and composed of a catalytic α subunit and β/γ regulatory subunits. It has been shown that intracellular AMP inhibits the KCa3.1 channel activity by AMPK activation. It has been also shown that the AMPK γ subunit interacts with a leucine zipper domain in the C-terminal region of the KCa3.1 channel (region 380-400) [43]. It is however unclear whether AMPK inhibits the channel by phosphorylating it (directly or through associated proteins), or by negative protein-protein interaction.

It has long been known that KCa3.1 channels are positively modulated by intracellular ATP, and the region 355-368 at the C-terminal, very close to the Ca²⁺-dependent CAMBD, is needed for this modulation [44]. Also essential for ATP modulation is the multibasic ¹⁵RKR¹⁷ motif in the N-terminal region (Fig. 2), suggesting that an interaction occurs between the N- and C-terminal of the channel [45]. Interestingly, it was later found that the 14 aminoacids required for the action of intracellular ATP (355-368) can bind the activated nucleoside diphosphate kinase B (NDPK-B) (Fig. 2), which in turn phosphorylates the histidine H358, resulting in increased activity of the KCa3.1 channel [46]. It was also found that, when dephosphorylated, the four H358 residues from the four channel subunits coordinate a Cu²⁺ ion resulting in channel inhibition, whereas their phosphorylation dislodges the coordinated Cu²⁺ and activates the channel [47]. Dephosphorylation of H358 and ensuing KCa3.1 channel inhibition are operated by the binding of histidine phosphatase (PHPT-1) [48]. Furthermore, NDPK-B is also a downstream effector of phosphoinositol-3-phosphate (PI3P), a membrane phospholipid continuously produced by the activity of PI3 kinases, quickly degraded by the PI3P phosphatase MTMR6 stably bound to the leucine zipper at the C-terminal region of the KCa3.1 (380-400), and known to activate KCa3.1 channels [49]. Altogether these data suggest that PI3P levels are carefully controlled in the immediate vicinity of KCa3.1 channels, in order to tune the needed KCa3.1 channel activity.

3.2. Modulation of KCa3.1 Channel Expression and Targeting to the Plasma Membrane

Besides gating modulation, regulation of the number of functional channels at the plasma membrane critically participates in setting the KCa3.1-mediated K⁺ efflux from the cell. Both AP-1 (Activator Protein 1) and REST (Repressor Element 1-Silencing Transcription factor) transcription factors have been found to regulate KCa3.1 channel expression. In T lymphocytes the ERK-1/2 pathway stimulates AP-1 and promotes KCa3.1 current and cell proliferation [50]. In accordance, in GL-15 human glioblastoma cells KCa3.1 mRNA levels were found to be positively modulated by the ERK-1/2 activity, that in tumoral cells is particularly high [7]. The second transcription factor found to modulate the KCa3.1 channel expression is REST. The *Kcnn4* gene contains two RE-1 (Restrictive Element 1) sites whose occupancy by REST represses gene transcription, and in vascular

smooth muscle cells downregulation of REST correlates with KCa3.1 channel upregulation [51].

Critical for the surface expression and tetramerization of the KCa3.1 subunits was also found to be the association of CAM to its CAMBD at the proximal C-terminal of the KCa3.1 channel [17]. It was later reported that the C-terminal leucine zipper domain is also needed for the correct folding of the channel C-terminal domain and the KCa3.1 channel trafficking to the plasma membrane [20, 52]. More recently also an overlapping leucine zipper/dileucine motif at the N-terminal of the channel has been implicated in channel tetramerization and trafficking [53]. Finally a critical role was found for intracellular L397 in KCa3.1 channel exit from the ER [54], and for a glutamate at S3 and two arginines at S4 for the correct folding of the S1-S4 domains [55].

To investigate the retrograde pathway used by KCa3.1 channels, [56] inserted a biotin ligase acceptor peptide (BLAP) at the S2-S3 loop, and found that KCa3.1 channels have a very short half-time in the plasma membrane (60-90 min), due to a very efficient internalization mediated by a C-terminal dileucine motif (Leu344/Leu345) [57]. After polyubiquitination and internalization KCa3.1 channels are shortly deubiquitinated before being targeted for lysosomal degradation via a rab-7 and MVB/ESCRT-dependent pathway [56, 58]. This very efficient degradation mechanism is likely needed to guarantee rapid changes in KCa3.1 plasma membrane expression, such as during its downregulation following the differentiation of C2C12 myoblasts to competent myocytes [59].

4. BASIC FUNCTIONS OF THE KCA3.1 CHANNEL

The KCa3.1 channel controls a number of basic cellular processes which translate in the modulation of several higher-order biological functions critical to brain tumors such as cell proliferation, migration, and apoptosis. The most relevant and widespread basic cellular processes controlled by KCa3.1 channels are the regulation of cell Ca²⁺ signaling and cell volume.

4.1. KCa3.1 Channel Regulation of Cell Ca²⁺ Signaling

In virtually all cells the stimulation of phospholipase C (PLC)-coupled membrane receptors triggers an initial IP₃-mediated release of Ca²⁺ from intracellular stores, followed by Ca²⁺ influx through store-operated Orai Ca²⁺ channels that are activated in response to Ca²⁺ depletion of the endoplasmic reticulum. One of the consequences of this massive Ca²⁺ influx, besides the activation of Ca²⁺-dependent ion channels, is membrane depolarization which, if left unchecked, would increasingly limit Ca²⁺ influx due to the reduced electrochemical driving force for Ca²⁺ ions. Notably, efflux of K⁺ ions following KCa3.1 channels activation by Ca²⁺ influx would oppose this dampening mechanism by hyperpolarizing the membrane towards the K⁺ equilibrium potential, and increasing the electromotive force on incoming Ca²⁺ (Fig. 4). Thus, the Ca²⁺ dependent gating of KCa3.1 channels is particularly well suited for this role, since the coupling of Ca²⁺ influx with a K⁺ channel activation will establish a positive feedback whereby Ca²⁺ influx will in-

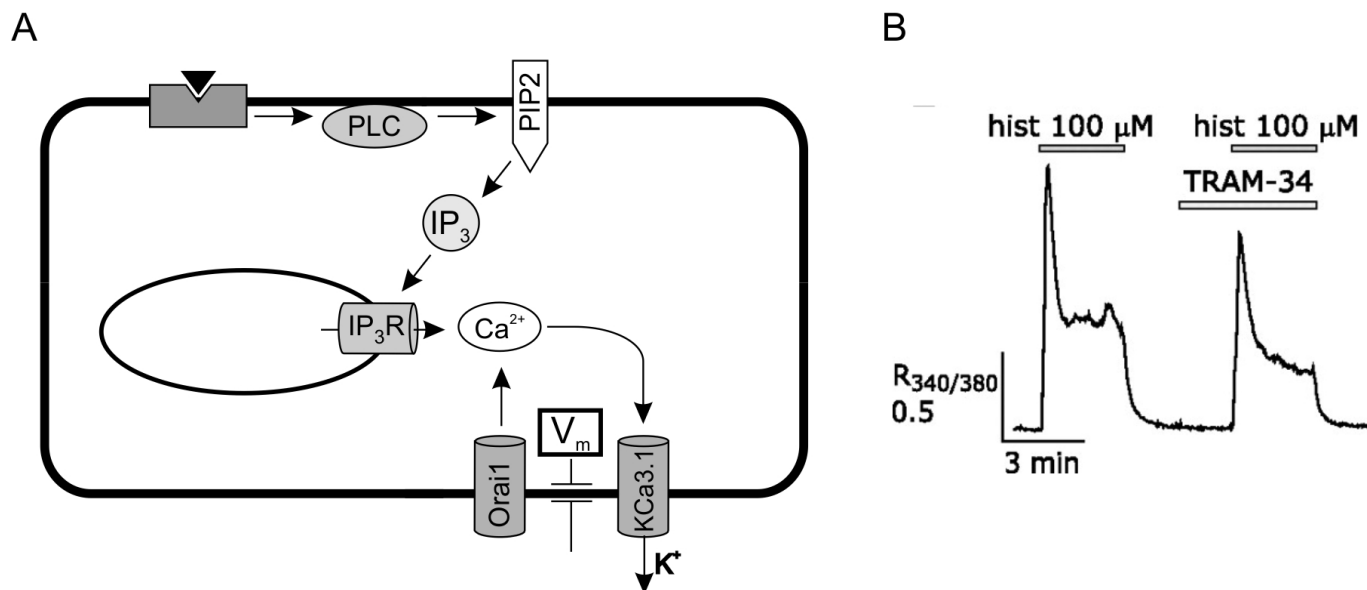


Fig. (4). **A**), Schematic of the role of KCa3.1 channels activity in controlling intracellular Ca²⁺ signals promoted by PLC-coupled receptors. Description in the text. **B**), Biphasic Ca²⁺ response upon prolonged application of histamine on a GL-15 glioblastoma cell, and effects of TRAM-34 on the sustained component. From [68].

crease K⁺ efflux, hyperpolarize the membrane, and further promote Ca²⁺ entry, thus amplifying the signal transduction.

This paradigm has been demonstrated in human macrophages, where KCa3.1 channels have been shown to hyperpolarize the membrane and increase the driving force for Ca²⁺ ions during store-operated Ca²⁺ entry [60, 61], and in T cells, where KCa3.1 channels are rapidly upregulated following cell activation, and used to maximize Ca²⁺ influx [62, 50]. Activated T cells isolated from KCa3.1^{-/-} mice show in fact a reduced Ca²⁺ response to T cell receptor activation [63]. Also mast cells use KCa3.1 channels to hyperpolarize the membrane and increase Ca²⁺ influx following antigen-mediated stimulation [64, 65]. In this case, KCa3.1 channels have been found to physically interact with the Orai1 subunit, suggesting that they may be activated by the Ca²⁺ microdomain that forms close to the store-operated Ca²⁺ channel [66]. Activation by Ca²⁺ microdomains has been demonstrated for rat microglial cells, where the Orai1-KCa3.1 functional coupling could be interrupted by BAPTA but not by EGTA that has a Ca²⁺ binding rate 10 times lower than BAPTA. Only a physical coupling between Orai1 and KCa3.1, with a separating distance of few nanometers, would explain these results [67]. An increase of [Ca²⁺]_i consisting of a fast peak due to IP₃-driven release of Ca²⁺ from intracellular stores, followed by a sustained phase resulting from Ca²⁺ influx through store-operated CRAC channels was also shown to occur upon prolonged application of histamine on GL-15 glioblastoma cells (Fig. 4B; [68]). The enhancing role of KCa3.1 channel in sustaining the protracted influx of external Ca²⁺ was shown by the marked reduction of the histamine-induced [Ca²⁺]_i increase in presence of TRAM-34 (Fig. 4B). This observation could be significant with regard to the KCa3.1 channel contribution to glioblastoma cell migration exerted through modulation of Ca²⁺ signals. In U87

glioblastoma cells we also observed that the promigratory agent fetal calf serum (FCS) promotes IP₃-driven [Ca²⁺]_i oscillations that cyclically activate KCa3.1 channels during cell migration [69]. Using a modeling approach we found that a channel activity with the properties of KCa3.1 channels could modulate IP₃ driven [Ca²⁺]_i oscillations (it increased amplitude, duration and frequency of each Ca²⁺ spike). Moreover, under specific conditions, the KCa3.1 channel activity was necessary for the cell to generate [Ca²⁺]_i oscillations [70]. This observation would also explain old experiments showing that the KCa3.1 channel inhibition by ChTx abolishes the bradykinin-induced [Ca²⁺]_i oscillations in C6 glioma cells [71].

4.2. KCa3.1 Channel Regulation of Cell Volume

Regulation of cell volume is critical for carrying out several basic biological processes including cell proliferation, migration, apoptosis. Focus here is on the regulatory volume decrease (RVD), as the prototypical process that occurs upon cell swelling, and the involvement of KCa3.1 channels in the process.

Osmotic permeability of cell membranes to water is orders of magnitude (10³-10⁶) higher than permeability to ions (Na⁺, K⁺, Cl⁻). With the cell behaving as a nearly perfect osmometer, a decrease in extracellular osmolarity leads to a rapid water inflow and increase in cell volume. Cell swelling then activates RVD that brings the cell volume back near to its initial value. RVD is sustained by the combined efflux of K⁺, Cl⁻, and organic osmolytes, and the extrusion of osmotic water [72]. In the majority of cell types hypotonic swelling leads to [Ca²⁺]_i increase, with contribution of both intracellular stores and plasma membrane Ca²⁺ channels. By analyzing a number of different cell types [73] reached the conclusion that the Ca²⁺-dependence of RVD correlates with the associ-

ated Ca^{2+} -dependent K^+ efflux. Following molecular cloning of KCa3.1 channel, it was easily recognized that this channel is in most cases the one mediating the Ca^{2+} -dependent K^+ efflux associated to the RVD.

The first clear evidence of a major role for KCa3.1 on RVD came from experiments on cloned mIK1 KCa3.1 channels expressed on *Xenopus* oocytes [74]. It was observed that while control (uninjected) oocytes subject to hypotonic solution swelled without showing any RVD, mIK1-expressing oocytes displayed a marked RVD that was sensitive to KCa3.1 blocker clotrimazole and to Ca^{2+} chelating agents. Voltage clamped mIK1-expressing oocytes subject to hypotonic swelling also showed outward Ca^{2+} -dependent currents that were clotrimazole-sensitive, and reversed near the K^+ equilibrium potential. It was subsequently found that KCa3.1 channels play a prominent role in the RVD response of several different cell types [20, 75-79].

In glioma C6 cells a gradual decrease in osmolarity, more similar to the osmolarity changes that may be encountered *in vivo*, activated a Ca^{2+} -dependent current showing voltage-independence, weak inward rectification, and sensitivity to charybdotoxin, properties typical of KCa3.1 channels [80]. Notably, this current did not activate following sudden osmolarity changes, as commonly used in experiments. This result suggests that under physiological conditions KCa3.1 channels may participate to the regulation of glioblastoma cell volume, as recently found for D54 glioma cells, where the apoptotic agent staurosporine induced a $[\text{Ca}^{2+}]_i$ increase, the activation of KCa3.1 channels, and a TRAM-34 sensitive volume reduction needed for the apoptotic induction [81].

CONCLUSION

We have illustrated the structural domains responsible for the gating of KCa3.1 channels and their modulation by several cytosolic molecules and proteins, a number of which physically interact with the C-terminal tails of the channel subunits. The role of KCa3.1 channel activity in two basic cell functions: the control of Ca^{2+} influx and the regulation of cell volume and shape, which have important consequences in higher-order cell functions such as migration and death, has also been highlighted. Hopefully, the increasing knowledge of the KCa3.1 channel structure/function relationship will provide the required basis for a beneficial modulation of channel activity under pathological conditions.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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