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Voltage-gated Potassium Channels (Kv7): Molecular Structure, Regulation, and Role in Pathogenesis

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Abstract

The potassium channels of the Kv7 family, comprising KCNQ1 to KCNQ5, assume a critical role in the regulation of membrane potential and cellular excitability within the nervous, cardiovascular, sensory, and endocrine systems. Their specificity to distinct tissues makes them associated with a wide range of pathological conditions, including epileptic encephalopathy, long QT syndrome, hearing impairment, and diabetes mellitus. The intricate molecular mechanisms regulating Kv7 channel function-including interactions with phosphatidylinositol 4,5-bisphosphate (PIP_2), calmodulin, and receptor-mediated signaling pathways-make these channels a key target for research in neuropharmacology and precision medicine. This review presents a comprehensive analysis of publications selected using specific keywords in international bibliographic databases (PubMed, Scopus, Web of Science, Google Scholar, and ScienceDirect) and specialized bioinformatics resources (PDB, UniProt, and AlphaFold). The focus is on research published between 2000 and 2025, with a particular emphasis on the past five years' findings. The final sample includes 128 original, peer-reviewed studies that meet criteria for scientific validity, innovation, and significance. The analysis uncovered the fundamental principles governing the regulation of Kv7 channels, encompassing lipid interactions, calcium-dependent modulation, and allosteric effects. Variations were observed among the Kv7.1-Kv7.5 isoforms in terms of their kinetics, the structure of the PIP_2-binding site, and susceptibility to pathological mutations. The Kv7.2/3 channels, which play a role in the generation of the M-current and the development of epilepsy, were examined in greater detail. Recent cryo-EM structural data were examined, along with preclinical and experimental efforts to pharmacologically modulate these channels using retigabine, ICA-069673, ZK-21, and novel derivatives. Consideration was given to the issue of tissue selectivity and the functional variability of pharmacological responses in the context of different mutations. The Kv7 ion channels constitute a sophisticated system that responds to stimuli from ions, lipids, and proteins. This intricate network enables precise regulation of neuronal activity and the maintenance of delicate balance. Recent breakthroughs in structural biology, genetic research, and electrophysiological studies have laid the groundwork for the development of targeted therapeutic approaches. However, there are still unanswered questions regarding the specific characteristics of various tissue types, the impact of post-translational modifications, and the consequences of mutations. Addressing these issues necessitates continued collaborative efforts to fully comprehend the intricate mechanisms underlying these phenomena.

Keywords: Kv7 channels; KCNQ; M-current; PIP_2; Calmodulin; Epilepsy; Channelopathies; Pharmacological modulation; Neuronal hyperexcitability.
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1. Introduction

The ion channels that belong to the Kv7 family constitute a group of membrane proteins that play a critical role in regulating the excitability and maintaining the stability of cell membrane potential. These channels can be found in the central and peripheral nervous Five distinct subtypes of the Kv7 (KCNQ) family have been identified: Kv7.1 through Kv7.5. While they share key structural domains, each subtype exhibits unique functional features. At the molecular level, Kv7 channels are characterized by a low activation threshold (approximately –60 mV), slow activation and deactivation kinetics, and a low propensity for inactivation. These

biophysical properties support Despite their electrophysiological similarities, Kv7 subtypes differ markedly in their functional specialization. This divergence arises from differences in subunit composition and tissupatterns. Kv7 subunits can form either homotetrameric or heteromeric channel complexes. For example, Kv7.2 and Kv7.3 co-assemble in neurons to generate the classical M-current, with distinct kinetics and pharmacological sensitivities compared to homomeric channels. Such diversity in channel assembly results in varied gating behavior and differential regulation by intracellular signaling cascades.Tissue-specific expression contributes to the versatility of Kv7 channels in different physiological contexts. Kv7.1 is expressed predominantly in cardiac myocytes, where it mediates the slowly activating delayed rectifier potassium current (IKs), which is essential for ventricular repolarization. In contrast, Kv7.2-Kv7.5 channels are mainly localized in neurons in both the central and peripheral nervous systems. They contribute to subthreshold potassium currents that help regulate excitability and prevent abnormal firing.Moreover, Kv7 channels are also found in non-excitable cell types, including epithelial, smooth muscle, and endocrine cells. Although their precise roles in these contexts remain to be fully elucidated, emerging evidence suggests that they may participate in processes such as ion homeostasis, secretion, and membrane potential regulation.[8,9]Kv7 channels play a crucial role in the somatic regions of neurons and the initial segments of axons, which are key areas for the formation of action potentials. The localization of Kv7 channels in these areas is essential for controlling the generation of pulses, as it is where the depolarizing currents from dendrites converge and the decision to initiate an action potential is made.Kv7 channels can activate at low membrane potentials and resist inactivation, creating a stable hyperpolarizing current that prevents excessive depolarization and helps maintain the stability of a neuron's resting potential. This prevents excessive firing of action potentials and ensures the stability of neuronal activity. Functionally, Kv7.2 and Kv7.3 act as low-pass filters, limiting the response of neurons to weak or brief input signals. This Studies using high-resolution imaging techniques, such as heteromeric channels are dominant in the axon initial sensitivity to intracellular regulators, such as calmodulin and PIP_2, as well as GPCR-dependent cascades.
As a result, thesechannels become key indicators of a cell's state.[11, 12] segments (AIS) of pyramidal neurons in the cortex and excitability, but they also actively participate in integrating metabolic and synaptic signals. This is due to their highMutations in the KCNQ genes not only affect the permeability of the cell membrane, but they also disrupt the functioning of various regulatory systems. This can lead to problems with cell function. Although the functional properties of Kv7 channels have been studied in detail, their spatial structure, particularly in cytoplasmic domains, is still poorly understood. This is a significant challenge, as most known mutations associated with channelopathies occur in these areas. The lack of information about structural organization makes it difficult to develop effective treatments and understand mutagenesis mechanisms. In this review, we will discuss recent developments in the molecular physiology and pathophysiology of Kv7 potassium channels. We will focus on their structure, regulatory mechanisms, genetic abnormalities, and pharmacological approaches to modulating these channels. The aim of this review is to organize available knowledge and identify areas that could contribute to the development of effective treatments for diseases caused by Kv7 channel dysregulation.

2. Methodology

This review is based on a systematic analysis of scientific literature, carried out in accordance with the principles of transparency, reproducibility, and scientific rigor. The primary sources of information included leading bibliographic and full-text databases such as PubMed, Scopus, Web of Science, ScienceDirect, Google Scholar, SpringerLink, and Wiley Online Library. The search strategy included a combination of keywords and terms: *Kv7* channels, KCNQ, M-current, *PIP2* regulation, calmodulin, epilepsy, channelopathy, pharmacology, structural biology, cryo-EM, long QT syndrome, KCNQ mutations, precision therapy.

The temporal scope of the literary sources under examination was predominantly confined to the period spanning from 2000 to 2025. The focus was primarily on the most noteworthy and pertinent research conducted within the past five to six years, specifically from 2019 to 2025. Nonetheless, some publications dating back to 1996 and 1999 have been incorporated due to their critical significance in elucidating the molecular underpinnings of Romano–Ward syndrome and identifying mutations in the KCNQ1 and, availability of experimental or clinical data on the molecular structure, regulation, and function of Kv7 channels; (ii) study as, sensorineural hearing loss, etc.; (iv) pharmacological studies aimed at modulating Kv7 activity; (v) Structural work, including cryo-EM, modeling and analysis of lipid of their

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posters and abstracts); (ii) reviews with outdated or incomplete data without proper critical evaluation; (iii) KCNQ2 genes, which are linked to channelopathies. The analysis included articles that meet the following criteria:(i) involvement in the pathogenesis of diseases of the nervous, cardiovascular and sensory systems; (iii) description of KCNQ1–5 mutations associated with epilepsy, arrhythmi interactions. The exclusion criteria covered: (i) materials without original data (*e.g.* letters to the editor duplicate publications and pre-registration versions without peer review; (iv) articles that do not contain sufficient information on the structure, physiology, or pathophysiology of Kv7 channels. Additionally, open bioinformatics resources, such as PDB, Uni Prot, and AlphaFold, were used to verify the spatial architecture and predict the effects of mutations on the molecular structure of Kv7 subunits. A total of more than 520 sources were reviewed and pre-evaluated. Of these, 128 publications that met the above criteria were included in the final review.

3. Structure and physiological role of Kv7 channels

The structures of all Kv channel family members exhibit significant similarities. In particular, it has been established that four α-subunits, referred to as Kvα, are essential for the functional assembly of Kv7 channels. Each subunit consists of approximately 650 to 940 amino acids.[13,14] Depending on the specific combination of Kva subunits within a Kv7 channel, different structural configurations can be formed. The resulting channels may be homomeric, if all four subunits are identical, or heteromeric, if they consist of two or more distinct Kvα subtypes. each of the four α-subunits contains six transmembrane segments (S1-S6) and two cytoplasmic ends: the N- and C-end. These transmembrane segments differ in both their structure and their functional purpose. The segments S1-S4 form the voltage-sensitive domain (VSD), which responds to changes in the membrane potential. The S4 segment within this domain plays a crucial role in channel opening and closing, as it is highly sensitive to fluctuations in the membrane potential and acts as the main sensor. [15]The S5 and S6 segments, along with the pure loop connecting them that contains the conserved GYGY motif, form the first domain (PD). This domain ensures the passage of potassium ions. [16] It is this loop that acts as a selective filter, being able to recognize and allow only potassium (K^+) ions to pass, effectively separating them from other cations. This is shown in Fig. 1.[17, 18] It is known that the voltage sensor, represented by the S1-S4 domains, is covalently connected to the pore domain, formed by the S5-S6 segments, using the S4-S5 amphiphilic linker helix. This section plays a key role in transmitting conformational changes from the voltage-dependent sensor to the channel gate mechanism. The S4–S5 linker interacts with the intracellular end of the S6 segment, also known as the S6T region, as well as with the corresponding region of the neighboring subunit.[19-21] The channel is opened and closed due to the structural flexibility of the S6 segment, which often contains a conserved proline residue. This residue forms a "hinge" that allows the S6 segment to bend, leading to the opening of the lower gate of the channel.[22] Kv7 channels are believed to have two gates: an upper gate, which is located in the area of the selective filter (between segments S5 and S6), and a lower gate, which is formed by the internal sections of S6 that are involved in channel activation and deactivation.

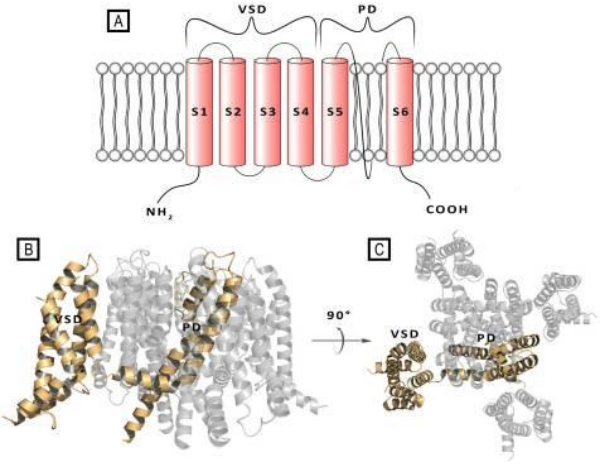

Fig. 1: Structure of Kv7 channels**.** (A) Schematic representation of one transmembrane subunit of the potassium channel Kv7, which consists of six alpha-helical segments (S1-S6). Segment S4 is responsible for voltage perception, while segments S5 and S6 form the pore domain. (B) Front view and (C) top view of the crystal structure of the Kv7.2 channel, recreated based on cryo-electron microscopy data (PDB ID: 7CR0). The figure shows a tetrameric assembly of subunits, one of which is highlighted in wheat color. The structure illustrates the spatial arrangement of transmembrane domains, which are arranged symmetrically around a central pore. Reproduced from.[17,18]

The upper gate is composed of a P-loop containing the GYGD motif, which forms part of the upper selective filter.[23] The lower gate consists of parts of the S6 helices that are interconnected to close the central opening of the channel. Despite the presence of two types of gates in the channel, the lower gates are responsible for activating it, as they directly respond to external stimuli such as changes in membrane potential.Kv7 channels operate within cells as large macromolecular complexes.[24] The α-subunits serve a structural role, forming the ion-conducting pore, while β-subunits modulate the properties and activity of the α-subunits in concert with other regulatory proteins.[25] The assembly of such a multi-component complex underlies the functional and structural diversity of Kv7 channels.

The activation of Kv7 channels exhibits three distinct functional states: a quiescent state, an activated state, and an

inactivated state. Similar to other ion channels, these channels possess two fundamental characteristics: an opening mechanism, which allows the channel to respond to appropriate stimuli, and selectivity, determining which ions can traverse the channel. While these attributes can be examined individually, they are intricately intertwined in the operational cycle of these channels. The structural alterations that occur during channel opening directly influence the passage of ions, a process known as channel activation. During the process of cellular depolarization, an increase in electrical potential within the Kv7 channel triggers a conformational shift, transforming the channel into a more energetically efficient open state. This process of channel activation is critical for the proper function of Kv7 channels.[16]

If the cell membrane remains depolarized for a prolonged period of time, most Kv channels will enter an inactivated state. Kv7 channels can be inactivated in two ways: rapidly (N-type) or slowly (C-type). Rapid inactivation (N-type) occurs when an inactivating peptide binds to the N-terminal region of the α or β subunit through a linker. This peptide has a globular structure.[15-16]

This spherical peptide molecule inserts itself into the open ion channel, effectively obstructing the flow of ions and momentarily halting their passage. In the context of slow inactivation, which is of type C, the P-loop within the selective filter assumes the role of an additional gate positioned nearer to the extracellular side of the membrane. This P-loop serves as a further barrier, effectively shutting down the ion channel and preventing the entry of ions.[26] Following inactivation, the ion channels undergo complete closure as the membrane potential declines to the resting level. It is worth noting that the scientific community at present lacks a unified view on the mechanisms underlying the activation of Kv7 channels, which remain understudied from both a biophysical and a physiological standpoint.

The carboxyl-terminal transmembrane domains of Kv7 potassium channels engage in intricate interactions with a variety of regulatory proteins and ligands, which are essential for their proper development and functioning. These include phosphatidylinositol-4,5-bisphosphate (PIP2),[27] calmodulin (CaM),[28] syntaxin 1A,[29] A-kinase anchoring proteins,[30] protein kinase C and ankyrin G-protein, among others.[31] Among these components, it is worth highlighting those that are of particular importance for Kv7 channels.

4. The M-current as a physiological manifestation of Kv7 channel activity

The M-current, which is produced by the Kv7.2 and Kv7.3 potassium channels, is a crucial component of the intricate system of neuronal control mechanisms that regulate subthreshold excitability. Its distinctive characteristics, such as its slow activation, resistance to inactivation, and relatively low threshold, make it a powerful tool for preventing spontaneous depolarizations and effectively filtering out synaptic noise. However, contrary to the previously held view of M-current as a purely inhibitory factor, recent research has shown that it can perform more complex and dynamic functions in the processing and modulation of incoming signals.

The majority of Kv7 channel subunits generate potassium currents that activate gradually, resembling the well-characterized M-current. These channels are distinguished by a notably hyperpolarized activation threshold, typically ranging from approximately -60 mV to as low as -80 mV in some cases. Moreover, the resulting currents do not exhibit inactivation.[32] The exceptions are homomers of Kv7.1, which exhibit a moderate degree of inactivation, and complexes of Kv7.1 and KCNE3, which are inherently in an open conformation.[33] The Kv7 channels are activated when the membrane potential approaches the resting level, and they exhibit slow kinetics. This property contributes to the stabilization and maintenance of the resting state of the membrane, effectively regulating neuronal excitability.[34]

Suppression of Kv7 channel activity induces a depolarized neuronal state, characterized by a reduction in rheobase and an increase in input resistance. Conversely, activation of these channels promotes neuronal hyperpolarization, thereby stabilizing membrane potential and decreasing the likelihood of action potential generation. These effects are illustrated in Fig. 2, which shows how the activity of Kv7.2 and Kv7.3 channels is modulated by retigabine and XE991, influencing the steady-state membrane potential.[35,36]

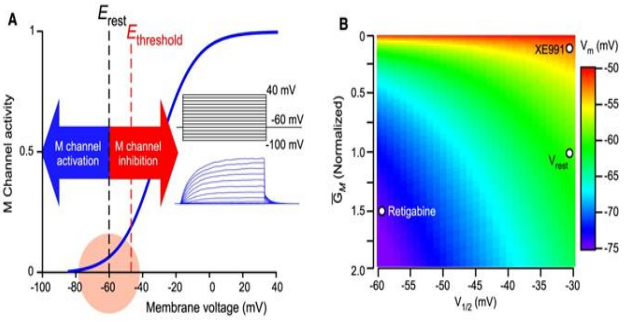

Fig. 2: Kv7 channels and neuronal excitability. (A) Voltage dependence of a heteromeric Kv7.2/Kv7.3 "M channel" in relation to the resting membrane potential and firing threshold of a representative somatosensory neuron. Inset: current traces recorded from a CHO cell overexpressing Kv7.2 and Kv7.3; the voltage protocol is shown above the traces. (B) Simulation of the effects of pharmacological modulation of Kv7.2/Kv7.3 channel activity by the opener retigabine and the blocker XE991 on the steady-state membrane potential (Vm). Modulation of M-channel voltage dependence and maximal conductance (G_M) in accordance with drug effects results in Vm shifts; leak currents were held constant. Panels (a) and (b) are Reproduced from.[35].

Historically, the M-current was first described in sympathetic neurons as potassium current modulated by muscarinic receptor activation.[37,38] It was later identified in structures of the central nervous system.[39,40] The subsequent discovery of the molecular basis of the M-current - specifically

the involvement of Kv7.2 and Kv7.3 subunits - enabled the integration of electrophysiological and molecular data.[41] These channels have since been shown to be particularly abundant in pyramidal neurons of the cortex and hippocampus, where they are primarily localized to the soma and proximal dendrites. In these regions, they effectively shunt excitatory input and serve as a form of negative feedback regulation.[42]

The M-current has a physiological significance that goes beyond just stabilizing cells. Eguchi *et al.*'s work[43] showed that Kv7.2/3 channels have inductive-like kinetics, which causes a frequency resonance between 4-12 Hz. This means the M-current is part of neural resonance - the ability for neurons to respond to specific frequencies without the need for traditional inactivating currents. Kv7 channels have activation and deactivation delays that mimic the behavior of an inductor. This allows them to filter out sinusoidal stimuli, but it's important to note that this research was done in HEK293 cells, which aren't fully representative of how ionic currents integrate in neurons in vivo. So the main question is: how much do the inductive properties of the M-current show up in real-life neurons, especially in structures with rhythmic activity like the hippocampus?

The maintenance of rhythmic activity and transitions between different states in the network is also regulated by the M-current. Della Porta *et al.* showed that blocking the M-current results in an elongation of up states and an increase in neuronal activity in the cortex.[44] These findings were replicated in biophysical models, which showed that reducing the M-current's density leads to an increase in active phase duration and synchronicity. This study is valuable because it integrates modeling and in vitro data. However, it was conducted using sections of the cortex that were anesthetized, which limits the ability to draw conclusions about how the M-current functions in the awake brain. Further research is needed to confirm these findings in vivo, taking into account interactions with HCN and Ca^{2+} currents.

From a molecular perspective, the functioning of the M-current depends on the coordinated activity of the voltage-gated domain (VSD) and the pore. A study by Edmond *et al.*, using voltage-clamp fluorometry, found that the movement of the S4 segment in Kv7.2 is closely linked to channel opening. Mutations that disrupt this interaction can significantly alter the current kinetics, even if conductivity is not affected.[45] This study provides evidence that disturbances in both the temporal dynamics and amplitude properties of Kv7 channel activity may contribute critically to the pathogenesis of epileptic encephalopathy. However, the findings are limited by the use of artificially introduced mutations and a heterologous expression system. Further investigations are required to determine whether these effects are recapitulated in neurons carrying endogenous pathogenic variants.

The possibility of pharmacological and intracellular modulation of the M-current increases the therapeutic potential of this system. Specifically, SGK1.1, a neuronal isoform of serine/threonine kinase, can enhance current through Kv7.2/3 channels even in the presence of mutations associated with epilepsy. The effect is achieved by inhibiting the activity of the ubiquitin ligase Nedd4-2 and increasing the expression of channels on the membrane, which opens up opportunities for restoring the function of the mutant Kv7 complex.[46] However, it remains to be seen how effectively this regulation will work in the mature brain in vivo and whether any compensatory adjustments will occur through changes in the expression of other ion channels.

An additional confirmation of the control of the M-current can be found in the work of Lu *et al.*,[47] They showed that a number of small molecules can change the amplitude and kinetics of the current as well as the voltage-dependent hysteresis. Some compounds, like flupirtin and QO-58, enhance I_M, while others, such as bisoprolol and phenobarbital, suppress it. It is important to note that the modulation of hysteresis can influence the behavior of neurons when processing rhythmic information. However, it is still unclear how these changes affect neuro dynamics in vivo. Additionally, the drugs used in these studies have multiple targets, and it is difficult to attribute the effects solely to Kv7 without more selective agents.

The severity and characteristics of the M-current can vary depending on the type of neuron, the stage of development, and the physiological state. Although the role of Kv7.5 is still not fully understood, data from single-cell transcriptomics indicate that there is significant heterogeneity in the expression of KCNQ subunits, even within the same morphological type. These differences may be crucial for the formation of local filtering and resonance properties. The most significant clinical consequence of these variations is the difference in phenotypes associated with mutations in KCNQ2/3 genes. In benign neonatal epilepsy, the M-current function is only partially affected, while in KCNQ2-associated disorders, dominant negative mutations or a significant decrease in sensitivity to phosphatidylinositol 4,5-bisphosphate (PIP2) occur, severely disrupting the M-current.[48]

Although most research on the M-current has been conducted in a laboratory setting, there are still several aspects that require further investigation. Specifically, it remains unclear how the M-current interacts with other ion channels, such as HCN and Ca2+ channels, as well as slow Na+ currents. The role of the M-current in synaptic plasticity is also uncertain.

Preliminary studies suggest that Kv7 channels may play a role in memory formation and sensory adaptation, but there is currently no unified theory that explains how the M-current contributes to long-term modulation. To address these questions, a comprehensive approach that combines various scientific disciplines, from molecular biophysics to neurophysiology, is necessary.

5. Endogenous regulation of Kv7 channels

Kv7 channels are subject to subtle endogenous regulation through various intracellular mechanisms, which allows them to adapt to a wide range of physiological conditions. Unlike many other potassium channels, Kv7 isoforms exhibit a pronounced dependence on molecules involved in signaling pathways, such as phosphatidylinositols, calmodulin, G proteins, cyclic adenosine monophosphate (cAMP), and calcium ions. This interaction makes Kv7 channels essential regulators of neuronal excitability and vascular tone, as well as important targets in the development of diseases associated with hyperexcitability. The functional sensitivity of Kv7 channels to the levels of membrane PIP_2, ionic calcium, and calmodulin (CaM) is due to their unique structural architecture, including a C-terminal domain that interacts with CaM and specific PIP2 binding sites in the transmembrane region. This dual control system allows Kv7 channels to integrate lipid and ionic signals, changing the probability of channel opening in response to physiological stimuli.

One of the central components in the endogenous regulation of the M-current is the activation of receptors associated with Gq/11 proteins. These include muscarinic M1 receptors (M1AChR), angiotensin II receptors (AT1R), bradykinin, and purinergic P2Y receptors. When these receptors are activated, they trigger the activation of phospholipase C (PLC). PLC hydrolyzes the membrane phosphatidylinositol-4,5-bisphosphate (PIP_2) to form IP_3 and diacylglycerol (DAG). IP_3 then interacts with IP3 receptors in the endoplasmic reticulum, promoting the release of calcium ions.[49]

The subcellular localization of these receptors determines the contribution of calcium to the modulation of the M-current. In the neurons of the superior cervical ganglion (SCG), for example, the spatial organization of M1AChR and AT1R limits the effect of IP_3 on ER, while P2Y and B2 receptors are located near IP3 receptors, providing efficient calcium mobilization.

Mobilized Ca^{2+} activates both calmodulin (CaM) and neuronal calcium sensor-1 (NCS-1). CaM decreases the channel's affinity for phosphatidylinositol 4,5-bisphosphate (PIP_2), whereas NCS-1 promotes PIP_2 resynthesis via activation of phosphatidylinositol 4-kinase (PI4K), thereby establishing a dual inhibitory pathway. By depleting PIP_2 and reducing its binding affinity to the channel, this mechanism finely tunes neuronal excitability. Fig. 3 illustrates the molecular integration of receptor stimulation, lipid metabolism, and calcium-dependent regulation of M channels in the superior cervical ganglion (SCG).[50]

Additional information supports the important role of muscarinic receptors in causing convulsive episodes. In particular, activation of M1 receptors has been shown to contribute to the onset of epilepsy by inhibiting M-currents and changing the balance between excitatory and inhibitory neurotransmission. Administration of atropine, an antagonist, before seizure induction prevents them from occurring, highlighting the importance of M1-mediated pathways during the initial stage of induction. However, continued seizures likely rely on other mechanisms.

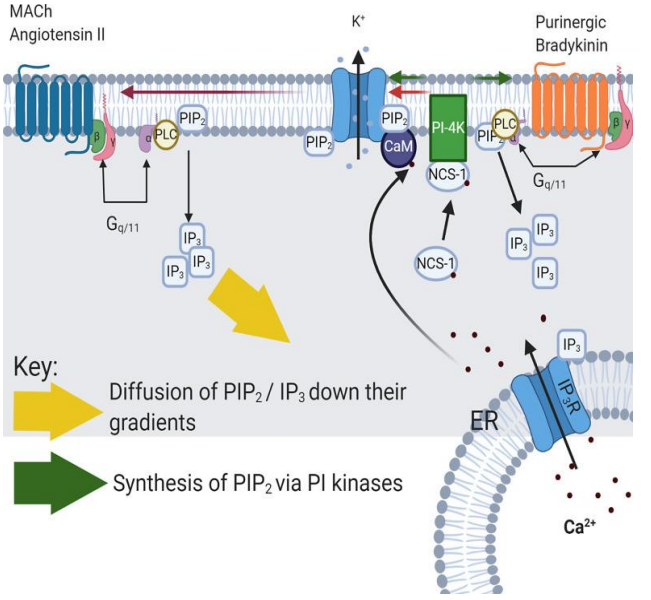

Fig. 3: Signaling cascades mediating inhibition of M channels in the superior cervical ganglion (SCG) via Gq/11 protein-coupled receptors. Pathways involving phospholipase C (PLC) activation, phosphatidylinositol 4,5-bisphosphate (PIP_2) hydrolysis, and inositol 1,4,5-trisphosphate (IP_3) formation are depicted. The yellow arrow indicates the diffusion of PIP_2 cleavage products, while the green arrow represents PIP_2 resynthesis catalyzed by phosphatidylinositol 4-kinase (PI4K) under the influence of neuronal calcium sensor-1 (NCS-1). The roles of Ca^{2+}, calmodulin (CaM), and IP_3 receptors within the endoplasmic reticulum (ER) are also illustrated. Reproduced from.[50]

Calmodulin (CaM) plays a crucial role in the regulation of Kv7 potassium channels. It acts as a structural stabilizer for the C-terminal domain of the channel and as a sensor for calcium ions. Calcium-dependent changes in the conformation of CaM alter its interaction with the channel. This can lead to either an increase or decrease in current, depending on the specific Kv7 isoform and the tissue in which it is expressed. Additionally, CaM is necessary for the proper transport of Kv7 channels from the endoplasmic reticulum to the cell membrane, ensuring that they are properly positioned for function.

One of the key regulators of Kv7 channel activity is phosphatidylinositol-4,5-bisphosphate (PI(4,5)P2), an important lipid co-factor necessary for the activation and stable functioning of all currently studied Kv7 subtypes. The presence of $PI(4,5)P_2$ is critically important for both the transition of the channel to the active state and maintaining its open state. Classical studies have shown that the activation of Gq protein-coupled receptors and the subsequent cleavage of $PI(4,5)P_2$ leads to rapid and reversible suppression of the M current generated by Kv7.2/Kv7.3 channels in neurons.[51] Similar regulatory mechanisms have also been

described for other Kv7 subtypes, such as Kv7.1 in cardiomyocytes, Kv7.4 in cochlear sensory cells, and Kv7.5 in vascular smooth muscle cells.[52-54]

Phosphatidylinositol-4,5-bisphosphate (PIP_2) binds to multiple sites at the interface between transmembrane segments and the voltage-sensing domain (VSD) of Kv7 potassium channels, stabilizing their open state. A deficiency in membrane PIP_2 leads to rapid channel closure, disrupting their function. Research based on directed mutagenesis and electrophysiological techniques has shown that different Kv7 channel isoforms vary in their dependence on PIP2 and affinity for this lipid co-factor. These differences likely determine the tissue specificity of the channels' sensitivity to signaling pathways associated with phosphoinositide hydrolysis.

From a pathophysiological standpoint, this implies that multiple regulatory nodes within the signaling network may serve as potential points of vulnerability. This concept was experimentally validated in the study by Zhang *et al.* (2015), which demonstrated that activation of NMDA receptors in corticotropin-releasing factor (CRF)-expressing neurons of the central amygdala (CeA) leads to inhibition of the M-current via two distinct molecular mechanisms.[55].

Short-term stimulation leads to a rapid decrease in current flow through Ca2+ -dependent activation of PI3K, which results in the depletion of PIP2. CaM and PKC are not involved in this process. Prolonged stimulation causes persistent current suppression through a PKC-dependent mechanism. This suppression is accompanied by a reduction in the expression of Kv7.3 on the membrane, while the level of Kv7.2 remains constant. This example demonstrates the subunit-specific and time-dependent modulation of channels during stress-induced neuroplasticity.

The separation by time and subunit composition illustrates the principle of functional allostery in the Kv7 structure. The Kv7 channel is not a fixed unit, but a dynamically assembled complex with multiple levels of sensitivity to intracellular signals. This feature is crucial for understanding pathological conditions and developing selective pharmacological modulators. However, the question remains about how stable these differences are in the face of chronic stress and inflammation.

Expanding the analysis of tissue specificity, relevant observations can be drawn from smooth muscle cells. In this context, vasopressin induces depolarization and an increase in intracellular Ca^{2+} concentration through inhibition of Kv7.5 channels. Notably, inhibition of phospholipase C (PLC) attenuates the effect of vasopressin but does not alter the action of XE991, indicating the involvement of a PLC-dependent mechanism that extends beyond mere PIP_2 depletion.[56] This challenges the reductionist view of PLC as solely a «PIP_2-hydrolyzing» enzyme and suggests the existence of alternative signaling pathways.

At the molecular level, these concepts are supported by the study of Pant *et al.*,[57] which demonstrated that the interaction of PIP_2 with Kv7.2 involves multiple binding sites, including the S4–S5 linker, the AB linker region, and the pre-Helix A segment. Mutations at residues R325, R214, and K219 were shown to destabilize the open channel state by disrupting the coupling between the voltage-sensing domain (VSD) and the pore domain. These findings suggest that lipid binding is not merely a secondary modulatory factor, but an integral structural component of channel gating. However, it is important to note that many in vitro studies employ supraphysiological concentrations of PIP_2, and thus the data should be extrapolated to in vivo conditions with caution.

It is noteworthy that lipid-mediated mechanisms in Kv7 potassium channels are coupled with the regulation exerted by calcium and calmodulin. Kv7.1 exhibits a distinctive form of inactivation that hinges on the interaction between extracellular Ca2+ ions and Ca2+/calmodulin (CaM). These ions serve to stabilize the selectivity filter by interacting with the linker region between S2 and S3 segments, as well as with the α-helix A. This process gives rise to a two-stage inactivation phenomenon,[58] bringing Kv7.1 closer in nature to the C-type inactivation. However, it necessitates a reassessment of the conventional classification due to the involvement of CaM. It remains uncertain whether similar mechanisms apply to Kv7.2 and Kv7.3 as well.

Considering the isoform-specificity of CaM action, it is worth noting that for Kv7.4, CaM has been shown to act as an activation inhibitor by interacting with the EF3 domain and the S2-S3 site. Mutations in these regions eliminate the inhibitory effect of Ca^{2+}/CaM, accelerating channel opening.[59] This suggests that the role of CaM is not universal, and depends on the specific isoform and cellular context. In sensory systems, such as hearing, this could be critical for developing therapeutic approaches.

The subunit composition also influences the sensitivity of the channel. Studies using concatemers and E140R mutations in Kv7.2 have demonstrated that even a single active subunit can induce partial conduction, indicating the allosteric nature of gating.[60] This explains the high sensitivity of the channel to heterozygous mutations. However, the question remains as to whether it is possible to pharmacologically compensate for defective subunits by enhancing the activity of neighboring ones.

Cryo-EM analysis of Kv7.5 has revealed two distinct PIP_2-binding sites - one located within the voltage-sensing domain (VSD) and another at the VSD-pore interface - that coordinate the channel's transition to the open state.[61] This topology is similar to enzymatic lipid regulation and may underlie mechanisms of allosteric modulation. However, it is unclear how these interactions are affected under pathological conditions. These findings are supported by an earlier study by Yang *et al.* (2019), which used high-resolution cryo-EM and structural modeling to investigate the conformational changes in human Kv7.5 when bound to calmodulin.[62] This study identified two distinct PIP_2-binding sites, one associated with the closed state and the other with the open state. The binding

of PIP_2 causes a structural rearrangement in the CaM-bound domain, highlighting the role of PIP_2 as a dynamic regulator. Calmodulin acts as a structural stabilizer, but also as a calcium-sensitive switch that regulates the transitions between the different functional states of Kv7.5.

The issue of post-translational regulation remains central, as modulation of Kv7.1 by calmodulin extends beyond channel gating to include control over channel trafficking. Mutations in calmodulin, such as G114W, impair channel expression and membrane localization.[63] These findings emphasize the importance of post-translational mechanisms, suggesting that mutant forms of calmodulin may have pleiotropic effects on other ion channels. Mandala and MacKinnon's study introduces a new perspective, demonstrating that changes in membrane potential modulate the accessibility of the PIP_2-binding site, influencing channel opening.[64] This suggests a mechanism of «electrosensory» tuning of lipid-protein interactions, but it still requires validation in vivo.

An additional dimension of modulation is revealed in the works dedicated to the role of G proteins. Of particular significance is the activation of Kv7.4 by the βγ subunits of these proteins, regardless of PLC or PIP_2.[65] This makes this pathway potentially useful in therapy for individuals with PIP_2 deficiency. However, questions remain regarding the possibility of selectively activating this pathway without unwanted side effects.

Recently, data has been obtained indicating the involvement of additional ionic and redox-modifying factors in this process. Specifically, zinc (Zn2+) can stabilize Kv7.2 in the open state, preventing the inhibitory effects of calcium (Ca2+) and calmodulin (CaM).[66] These results were confirmed in HEK293 cells and primary neurons, increasing confidence in the functional significance of this mechanism. In a study by Nuñez *et al.*,[67] it was found that redox modulation of the S2-S3 loop of Kv7.4 activates the channel through the participation of the EF3 CaM domain. This study used FRET (Förster resonance energy transfer) and NMR (nuclear magnetic resonance) spectroscopy methods, as well as electrophysiology on HEK293 cells. These techniques allowed us to investigate the structural changes in the CaM-Kv7.4 complex during oxidation and demonstrate the importance of calcium binding to the EF3 domain of CaM for the redox-sensitive activation of the channel. The results showed that the interaction between calcium and the EF3 domain is crucial for the functioning of the channel, confirming the idea of CaM as a redox sensor. Additionally, mutations in the S2-S3 region that modify electrostatic interactions with EF3 can selectively alter the channel's sensitivity to fluctuations in oxidative status, providing further support for this hypothesis.

In conclusion, it is important to consider the dynamics of the lipid environment when analyzing the behavior of Kv7 channels. Studies by Chen *et al.* and Kongmeneck *et al.* have shown that the movement of PIP2 between different sites within the channel is a dynamic process that influences the deactivation of Kv7.[68,69] The Kv7.1/KCNE1 complex requires two binding sites for PIP2, one of which is regulated by KCNE1. This unique arrangement of lipid molecules creates a specific "memory" or "inertia" in the channel's behavior.

During our scientific research into the study of Kv7 channels (AP19680470), we have come to the conclusion that these channels play a crucial role in the neuronal regulation process.

In our in vitro study of paroxysmal depolarization, we discovered that calcium-permeable AMPA receptors (CP-AMPARs) expressed in GABAergic interneurons play a crucial role in driving hyperpolarization and the subsequent disinhibition of glutamatergic neurons in the hippocampus. The application of the CP-AMPAR antagonist NASPM resulted in a prolongation of the post-depolarization hyperpolarization (PDH) bursts and a reduction in their amplitude. Similar results were obtained when using the Kv7 channel blocker XE991. This also promoted the prolongation of PDH clusters and attenuated the hyperpolarization response, as shown in Fig. 4. Conversely, activation of Kv7 channels by retigabine enhanced the hyperpolarization after depolarization and shortened the duration of both the depolarization and hyperpolarization phases of PDH, as seen in Fig. 5. These data highlight the close relationship between the neurotransmission process mediated by CP-AMPA receptors and the mechanisms for stabilizing the membrane potential controlled by Kv7 potassium channels.

Thus, Kv7 channels play a dual role in the brain: they not only limit excitability but also actively regulate neural activity, especially in situations of excessive arousal, such as during epileptic seizures. Thanks to their unique ability to integrate signals from various sources, including receptors, ions, lipids, and intracellular proteins, Kv7 channels act as multifunctional sensors that can detect changes in the cell's status. This makes them promising targets for therapies aimed at restoring normal electrical activity in brain networks that have been altered by disease or injury.

Kv7 channel regulation is a complex and dynamic process that involves many interrelated components. Each component, including pip2, calmodulin, beta-gamma G protein subunits, zinc ions, and membrane potential, plays a unique role in fine-tuning and coordinating channel activity. Together, these components form a flexible network that can be disrupted by various factors, leading to pathologies such as epilepsy, sensory disorders, and cellular homeostasis disturbances. In light of this, it is important to develop selective methods for modulating these interactions, especially when there are pathological changes in the lipid environment or calcium signal modulation. This could open up new possibilities for treating neuroinflammatory and neurodegenerative diseases. The intricate web of endogenous regulation governing Kv7 channels constitutes a dynamically harmonious network, where phosphoinositide lipids, calmodulin, calcium ions, G-protein subunits, and redox-regulating factors assume pivotal

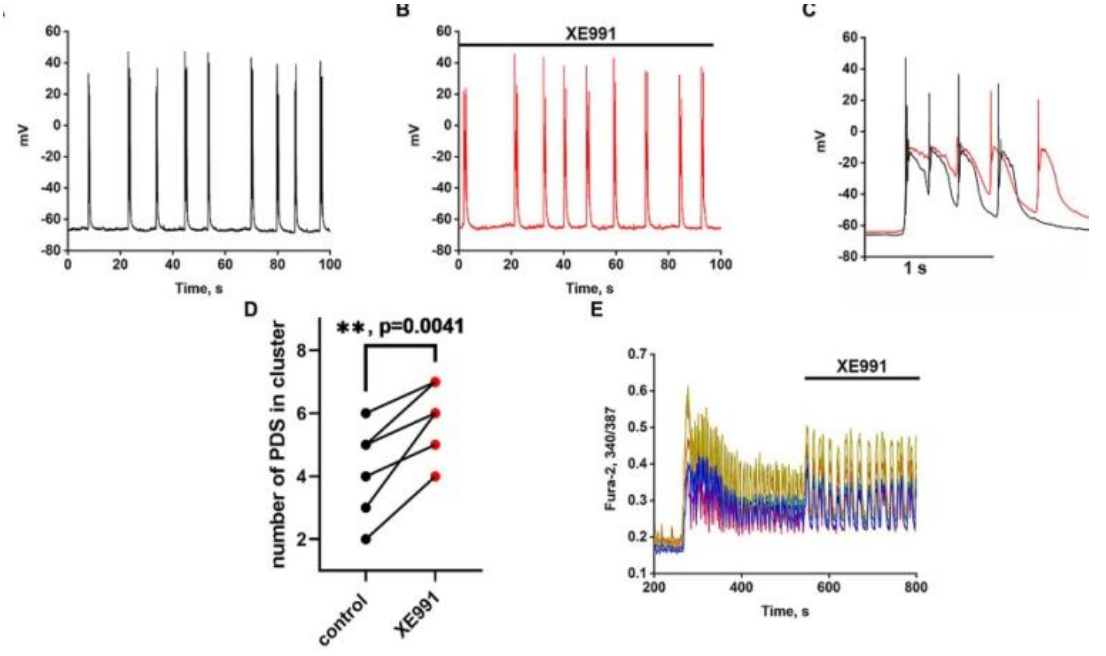

Fig. 4: Effect of the Kv7 blocker XE991 (10 μM). (A) Periodic AP bursts (PDS clusters) generated by a glutamatergic neuron in the presence of bicuculline in control. (B) Periodic AP bursts generated by the same neuron under the same conditions in the presence of the Kv7 blocker XE991; N = 4. (C) Comparison of two clusters: in control (black curve) and with the Kv7 blocker (red curve). The rate of hyperpolarization in the first PDS was 125 ± 2 mV/s in control, decreasing to 44 ± 2 mV/s with the blocker. The PDS clusters are from Fig. A and B. (D) Diagram showing the number of PDSs in clusters in control and with XE991. Each dot represents the mean number of PDSs before (black) and after (red) XE991 treatment. (E) Changes in [Ca2+]i in 8 random neurons after 8 mM NH4Cl and 20 μM XE991. The Kv7 channel blocker XE991 prolongs the duration of calcium (Ca2+) pulses from 6 to 10 seconds. N = 3 Reproduced from.[70]

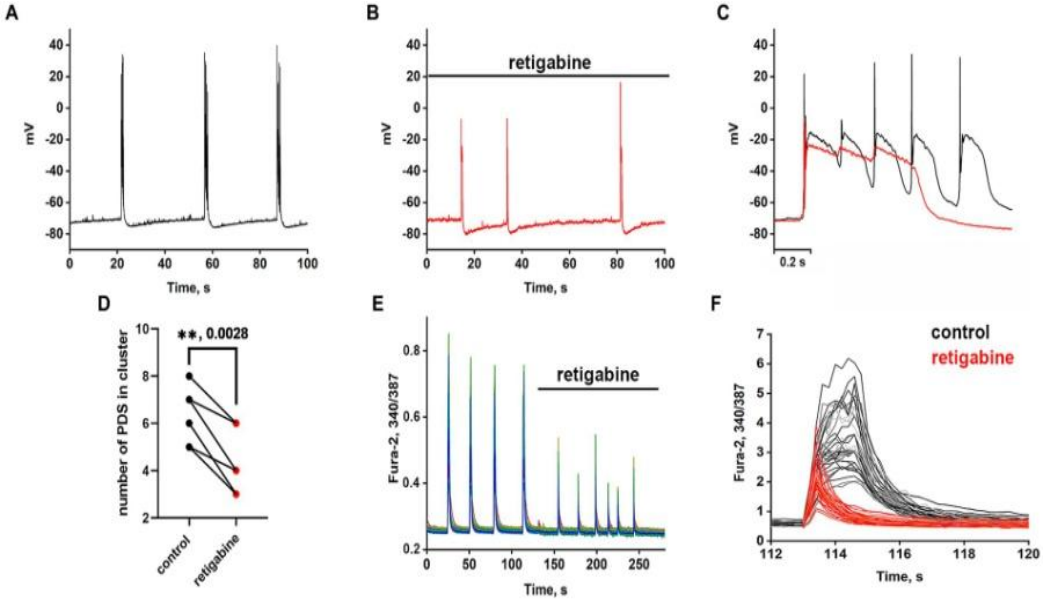

Fig. 5: Periodic PDS clusters in glutamatergic neurons during bicuculline-induced epileptiform activity in control (A) and after retigabine (2.5 μM) application (B). (C) Enlarged and superimposed PDS clusters in control (black curve) and in the presence of retigabine (red curve). (D) Diagram showing the number of individual PDSs in the cluster in control and after retigabine application; paired t-test. Each dot corresponds to the mean number of PDSs in individual neurons before (black dots) and after (red dots) retigabine application. (E) Spontaneous synchronized epileptiform Ca2+ pulses in control and after retigabine addition. The half-width of the pulses decreases from 2 s to 0.8 s in the presence of retigabine; N = 4. (F) Ca2+ pulses in control and in the presence of retigabine. Decrease in Ca2+ pulse duration after the addition of retigabine (from figure part label E). Black and red curves represent signals from the same neurons, before (black) and after the addition of retigabine (red). These neurons are all glutamatergic Reproduced from.[70]

roles. In recent times, there has been substantial progress in elucidating the intricate molecular interactions between phosphatidylinositol 4,5-bisphosphate (PIP_2) and diverse domains of Kv7. This includes elucidating the structural arrangement of lipid-binding sites, unraveling the role of calmodulin as a structural component sensitive to calcium, and discerning isoform-specific mechanisms of inactivation. These advancements have significantly deepened our comprehension of how cellular signaling is integrated and how Kv7 channels respond to dynamic physiological conditions.

Nonetheless, there are several unresolved matters of fundamental and practical significance. It is still not completely clear how chronic pathologies, such as inflammation, ischemia, and neuronal degeneration, affect the susceptibility of channels to regulatory stimuli, particularly in the context of prolonged deprivation of PIP_2 or impaired calcium homeostasis. The mechanisms by which mutations in the C-terminus of Kv7 or CaM disrupt allosteric signaling between the voltage-sensing domain and the pore region require further investigation at the cellular level. Moreover, the role of tissue specificity in shaping the effector response remains underexplored, particularly with regard to the stability of observed regulatory patterns in a heterogeneous lipid milieu in vivo.

Consequently, future investigations should concentrate on a comprehensive examination of the intricate interplay between lipids, proteins, and ions within living cells, employing cutting-edge techniques such as fluorescence resonance energy transfer (FRET), cryogenic electron microscopy (cryo-EM), optical sensors, and genetically manipulated models.

Only then will it become feasible to not merely elucidate individual mechanisms but also comprehend how Kv7 channels operate as context-specific sensors of cellular status. This knowledge is essential for the design of tailored therapeutic interventions aimed at addressing diseases linked to dysregulated ion and signalling homeostasis.

6. Pharmacological regulation of Kv7 Channels

Pharmacological modulation of potassium channels remains one of the most active and promising areas of research in the treatment of conditions associated with neuronal hyperexcitability. Specifically, channels such as Kv7.2 and Kv7.3, which form the M-current in the central nervous system, exhibit unique biophysical properties. They are activated at subthreshold potentials and do not inactivate. Their opening significantly increases the threshold for generating action potentials.

Even a slight reduction in M-current activity, such as that caused by mutations in KCNQ2/3, can result in clinically significant disorders, ranging from epilepsy to age-related sleep disorders. This has been demonstrated in an animal model of aging.[71]

One of the first clinically tested activators of these channels was retigabine. It has been shown to stabilize the open state of Kv7.2-Kv7.5 by interacting with the pore site, demonstrating its ability to activate these channels. Despite its anticonvulsant effects, its pharmacokinetics and side effects, including tissue pigmentation and bladder dysfunction, led to its removal from the market. This marked a shift in strategies, away from a broad range of activation profiles towards the development of more specific, molecularly targeted ligands. For example, compounds in the ICA series, such as ICA-069673, affect the voltage-sensor domain (VSD) and have tissue-specific activity. The work of Sun and Undem demonstrated that activation of Kv7.2/Kv7.3 by this compound reduces the excitability of visceral afferent fibers of the vagus nerve.[72] Importantly, the effect was completely lost in mice with KCNQ2/KCNQ3 deletions, emphasizing the high specificity and lack of off-target effects of this compound.

In modern pharmacology, special attention is paid to compounds that can restore the function of Kv7 ion channels, which have been mutated. These channels are responsible for neuronal arousal and their dysfunction can lead to various disorders. Lipophilic derivatives, such as N-arachidonoyl amine and N-arachidonoyl taurine, have been shown to normalize the activity of Kv7.3 channels with amino acid substitutions associated with impaired arousal. These compounds stabilize the active state of the voltage-sensing domain (VSD), partially compensating for the disruption of the allosteric interaction between the sensory and pore domains. This strategy of pharmacological intervention opens up the possibility of developing targeted molecules for precision treatment of inherited channelopathies.[73]

Of fundamental interest are the subtype-specific actions of polyunsaturated fatty acids (PUFA). In Frampton *et al.*,[74] it was shown that PUFA activates Kv7.5 but inhibits Kv7.4, despite their structural homology. This multi-directional effect is explained by differences in lipid-sensitive sites, and it opens up opportunities for the development of isoform-selective vasodilators and auditory protective agents.

Against this background, clinical attempts to modulate the activity of Kv7.2/3 in gain-of-function (GoF) mutations associated with epilepsy and autism are of particular interest. Donepezil, an acetylcholinesterase inhibitor, has been tested as a potential treatment for these conditions. It is believed to act as an M-current inhibitor, capable of normalizing neuronal activity in patients with KCNQ2/3 gain-of-function mutations. Clinical trials have shown that the use of donepezil can lead to improvements in cognitive functions and reductions in the severity of autism symptoms.[75] However, it is important to note that these findings are based on limited sample sizes and further research is needed to fully understand the effects of this treatment.

It is worth noting that certain psychedelic substances, which interact with serotonin receptors, have the ability to modulate the M-current. Specifically, the compound 25CN-NBOH has been shown to reduce the excitability of prefrontal cortical neurons by activating Kv7 channels. This effect occurs independently of the well-established action of this

compound on 5-HT2A receptors, as demonstrated in a study by.[76] These findings suggest the existence of "off-target" modes of action for psychotropic molecules, and emphasize the importance of Kv7 channels in neuronal activity regulation.

In the context where mutations affect the pore-forming region of KCNQ2, the activation of this channel by pharmacological ligands, such as retigabine, HN37, and XEN1101, can partially restore the current even in cases with severe structural abnormalities. This observation underscores the significance of functional mutation analysis for the design of targeted therapeutic interventions.[77]

Investigations into the mechanism of action of Kv7 inhibitors are particularly noteworthy. One such inhibitor, ML252, has been identified as a selective inhibitor of Kv7.2/Kv7.3 channels, acting through the pore region. A study by Kanyo *et al.* revealed that a tryptophan residue (W236 in Kv7.2 and W265 in Kv7.3) is crucial for the binding of ML252.[78] The substitution of this residue significantly diminishes the sensitivity to the inhibitory effect of ML252. Interestingly, this site is also involved in the binding of retigabine and ML213, indicating a shared binding site for activation and inhibition. This competitive interaction makes ML252 a valuable tool for both fundamental research and modeling the effects of modulation at the pore level.

In addition, a previously unreported chemotype, ZK-21, derived from 4-aminotetrahydroquinoline, has been identified in recent research using the automated high-throughput patch-clamp screening platform (SyncroPatch). This compound interacts with Kv7.2 channels through the W236 residue at a different stereochemical angle than retigabine, modulating their activity. The unique binding mechanism of ZK-21 opens up the possibility of developing more selective therapeutic agents with reduced risk of unwanted side effects.[79]

Compound 60 is a chemically stable version of retigabine. It was made based on the crystal structure of the drug. Musella *et al.*[80] found that it can reduce seizure activity in mice when they used the PTZ test. This compound has better blood-brain barrier penetration and photostability than the original drug. It also doesn't cause skin irritation, so it's a good candidate for treating epilepsy that doesn't respond to other treatments. But it hasn't been tested in humans yet, so more research needs to be done before it can be used in clinics.

Alongside the central nervous system, pharmacological modulation of potassium channels from the Kv7 family also affects other systems. For example, in the vascular system, activators of Kv7.4, such as URO-K10, have vasodilatory properties and can help protect against hypertrophic changes in people with pulmonary hypertension.[81] Likewise, in cardiac tissue, activation of Kv7.1/KCNE1 by ARA-S stabilizes ventricular repolarization and reduces the QT interval,[82] which is linked to lipid sensitivity. These effects emphasize the need for targeted molecules that act on specific isoforms to achieve desired outcomes.

The analytical review conducted by Perucca and Taglialatela underscores the fact that efforts to create drugs targeting Kv7.2/7.3 have been increasingly concentrated on enhancing isoform specificity and minimizing adverse effects.[83] Compounds such as azetucalner, pinegabine, BHV-7000, and CB-003 have demonstrated promising outcomes in preclinical models of refractory epilepsy. Nonetheless, their clinical effectiveness necessitates validation through larger-scale investigations.

Based on the analysis conducted, it is possible to conclude that the pharmacological modulation of potassium channels Kv7 represents a dynamically evolving field within neuropharmacology, holding significant promise for the development of precision medicine.

The progression from nonspecific activators towards structurally based isoform-specific compounds signifies the maturation of this area. Despite the advancements made in the development of activators and inhibitors, there remain several critical challenges. These include a deeper understanding of allosteric mechanisms of activation and inhibition in mutant channel variants, as well as accurate prediction of the efficacy of novel compounds in vivo experiments.

Moreover, further investigation is required in areas such as tissue and subcellular specificity, interactions with lipid milieus, and potential interactions with intracellular regulatory mechanisms.

Thus, at this juncture, the field of Kv7 pharmacology finds itself at the nexus of fundamental biophysics, molecular medicine, and clinical neurogenetics. Only at this convergence of these domains is it possible to devise therapeutic strategies that can effectively manage hyperexcitability while minimizing adverse effects and achieving a high level of personalized care.

The pharmacological modulation of Kv7 potassium channels remains a subject of intense research in the field of medicine, particularly in the context of conditions characterized by excessive neuronal excitability. In recent years, there has been a paradigm shift in the treatment approach, moving away from the use of broadly activating agents like retigabine and towards the development of more focused, isoform- and tissue-specific molecules.

This shift is based on a deeper understanding of the structural features and pathophysiology of these channels. One notable achievement in this area is the expansion of therapeutic options beyond nonspecific channel activation towards restoration of function in specific KCNQ2/3 channel mutations. This has been made possible through the development of novel compounds with enhanced bioavailability, lipid solubility, and safety profiles, such as ICA-069673, ZK-21, and compound 60, which demonstrate the promise of personalized neuropharmacological approaches.

Another notable advancement was the discovery of gain-of-function mutations, which necessitate the use of inhibition rather than activation for channel modulation. This discovery has paved the way for the pursuit of targeted antagonists, an area of research that holds great promise.

Advanced methodologies, such as high-performance electrophysiological testing, cryo-electron microscopy simulations, and docking algorithms, have revolutionized our understanding of the interaction between ligands and sensory and pore-forming domains. This has not only improved the efficacy of compounds but also reduced the likelihood of systemic side effects. By incorporating information about the sensitivity of mutant channels to pharmacological intervention, we have expanded the possibilities for personalized treatment in hereditary channelopathies.

However, there are still unresolved issues that hinder the clinical implementation of the accumulated knowledge. One of these is ensuring the specificity of the compounds being developed for both isoforms and tissues. Although the development of molecules such as ICA-069673 and ZK-21 has improved the specificity of action, the risk of systemic side effects remains a concern.

Even when a mutation is accurately identified, predicting the therapeutic response remains challenging. The same ligand can interact with different mutant forms of the channel in different ways, depending on the mechanism, whether it is a loss of function, an enhancement, or a dominant negative effect. This functional ambiguity necessitates preliminary in vitro screening, particularly in rare cases.

Another obstacle is the scarcity of clinical data on novel compounds, particularly in cases where pharmacological interventions are employed in patients with confirmed KCNQ2/3 mutations. It is crucial to exercise caution when extrapolating preclinical findings to actual clinical practice.

Moreover, the long-term effects of drugs on cellular adaptability, transcriptional regulation, and equilibrium have not been thoroughly investigated. This is particularly relevant in the context of long-term treatment, where the effects of drugs can accumulate over time.

Furthermore, the intricate interactions between Kv7 channels and regulatory factors such as PIP2, calmodulin, cholinergic, and serotonergic systems contribute to the biological variability. These interactions can either enhance or neutralize the effects of a drug, depending on the functional context and tissue type. A deeper understanding of these interactions could lead to improved therapeutic outcomes and increased predictability.

In the coming years, it will be essential to adopt a more organized strategy for drug creation. This will entail shifting from precise structural docking experiments to phenotypic screening and utilizing a multi-level biological model that encompasses cellular, synaptic, and behavioral levels.

Only a multidisciplinary approach, involving the collaboration of neurophysiologists, pharmacogeneticists, structural biophysicists, and clinical neuroscientists, will guarantee the development of truly effective and secure medications for addressing Kv7-related hyperexcitability.

7. Kv7 channelopathies: from molecular dysfunction to clinical phenotype

The expression of Kv7 channels depends on the type of tissue. These channels perform specific functions in different tissues. For example, Kv7.1 is mainly expressed in cardiomyocytes, where it forms a slow rectifying potassium current (IKs) together with the KCNE1 subunit. This current is crucial for the repolarization of heart ventricles.[4] Kv7.1 can also be found in other tissues, such as the thyroid gland, lungs, gastrointestinal tract, small intestine, pancreas, brain structures, and ovaries. However, its functions in these tissues are not well understood.[7-8]

The Kv7.2 and Kv7.3 channel-forming subunits, predominantly expressed in neurons of the central and peripheral nervous systems, form heterotetrameric complexes that give rise to the so-called M-current (IKM). This current reduces the excitability of neurons, helping to stabilize the membrane potential.[41]

Kv7.4 plays a significant role in auditory function, regulating the internal excitability of outer hair cells in the cochlea.[84] In addition to this, it is expressed in vascular and visceral smooth muscles, participating in maintaining basal tone and responding to myogenic stimuli.[85] Kv7.4 also contributes to the processes of proliferation, differentiation, and response to damage in skel *et al* muscle cells.

Kv7.5 is predominantly found in neurons,[86] but it is also expressed in skel *et al* and smooth muscles.[85] This indicates a wide range of physiological functions for this protein.

Mutations in the KCNQ genes that encode Kv7 channel proteins can cause a number of hereditary disorders. Such mutations can alter the electrophysiological properties of cells, disrupting signal transmission in various organs and tissues. This leads to the development of diseases affecting the cardiovascular, nervous, and sensory systems, including long QT syndrome (LQTS), benign familial neonatal seizures (BFNC), autosomal dominant sensorineural hearing loss (DFNA2), and other conditions caused by impaired cellular excitability.

8. Kv7.1 (KCNQ1)

Kv7.1, also known as KCNQ1, is a member of the Kv7 ion channel family. It forms part of a complex that is essential for regulating cellular excitability, particularly in cardiomyocytes. The gene encoding the potassium channel subunit of this complex was first identified through positional cloning in the beginning of our understanding of the molecular mechanisms underlying cardiac channelopathies. However, subsequent research has expanded our knowledge of Kv7.1's functions beyond the realm of the heart. It has been shown to play a role in the regulation of excitability in various tissues and organs beyond the cardiovascular system..

Structurally, Kv7.1 constitutes a tetrameric assembly. Unlike other members of the Kv7 family, it does not readily form heterotetrameric complexes with its counterparts. Instead, its functionality is primarily regulated by the β-subunit, KCNE1. In conjunction with this subunit, Kv7.1 gives rise to a slowly activating IKs current, which is essential for the later phase of cardiomyocyte repolarisation (Fig. 6).[87-89] The intricate molecular mechanisms underlying this interaction are gradually becoming more elucidated. Advanced techniques such as electron paramagnetic resonance (EPR), coupled with the use of directional spin labeling, have revealed that KCNE1 restricts the local mobility of specific regions of Kv7.1 through direct contact. This interaction stabilizes the functional conformation of the complex, thereby facilitating the necessary activation kinetics.[90] This dynamic allosteric interaction significantly diverges from the traditional paradigm that solely views functional disorders in terms of a loss of conductivity. Rather, it proposes a novel perspective that considers channelopathy as a result of disruptions in the structural integrity of the channel complex.

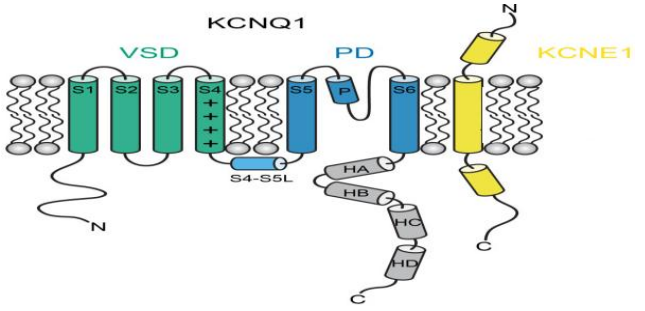

Fig. 6: Topological organization of the KCNQ1–KCNE1 channel complex**.** The KCNQ1 protein consists of three major domains: a voltage-sensing domain (VSD; helices S1–S4), a pore domain (PD; helices S5–P–S6), and a cytosolic domain (helices HA–HD), shown in green, blue, and gray, respectively. The KCNE1 subunit is represented by a single-pass transmembrane domain (TMD), flanked by intracellular and extracellular regions containing helical structures. Reproduced from.[87].

A crucial aspect in regulating the function of Kv7.1 involves the short transmembrane FTL motif within KCNE1, which has been demonstrated to be situated between the domain sensitive to stress and the pore region of the channel. This motif serves to delay the transition of the channel towards the open state, providing a molecular explanation for the distinctive kinetic properties of IKs.[87] Moreover, this interaction serves as the foundation for the pathogenesis of Long QT Syndrome (LQTS) (Table 1), particularly in cases where mutations disrupt this interaction. Nonetheless, there remains a need for further investigation into the influence of additional physiological factors such as lipid environment and calmodulin on the stability and dynamic behavior of the complex within the living organism. These elements may potentially impact the functioning of the Kv7.1 channel, potentially contributing to the development of LQTS.

The ion-dependent regulation of the Kv7.1 channel presents an intriguing aspect that has been previously underappreciated. The study conducted by Abrahamyan *et al.*[91] revealed that extracellular potassium ions can exert a partial inhibitory effect on the channel, binding to a specific filter and resulting in a reduction in the unitary conductance without completely obstructing the pore. This phenomenon assumes clinical significance in situations involving altered extracellular ionic conditions, such as ischemic or hyperkalemic states. It underscores the critical role of accessory subunits in modulating the channel's sensitivity to such alterations.

The functional disorders of Kv7.1, which are associated with mutations in the KCNQ1 gene, have long been associated with a loss of function and a prolonged QT interval. However, recent research by Brewer *et al.* has demonstrated that these clinical symptoms can arise from a variety of molecular mechanisms, including aberrations in transport, changes in channel dynamics, and the disruption of the KCNE1 complex.[92] One of the key challenges in this area is the limited ability of in silico methods to accurately predict the pathogenicity of genetic variations. These methods often fail to take into account the context of amino acid substitutions in transmembrane regions and interface regions.

At the molecular level, the study found that there is a critical constriction in the pore area at position G345, which prevents fully hydrated potassium ions from passing through. Ions cross this region in a partially dehydrated state, and mutations at this position reduce ion conductivity, which is associated with mild forms of channelopathies.[93] These findings allow us to reconsider the mechanism of loss-of-function» of Kv7.1 as a disruption of the pathophysiology of Kv7.1 closer to that of neuronal Kv7.2 and Kv7.3 channels.

The role of Kv7.1 extends beyond cardiac physiology. Clinical and experimental data, such as the case of Zhou *et al.* (2024), with the homozygous mutation R397W, indicate that Kv7.1 has a critical effect on the excitability of pancreatic β cells and insulin secretion.[94] This mutation leads to hypersecretion of insulin and subsequent depletion of secretory potential, highlighting the tissue-specific role of Kv7.1 and the complexity of phenotypic manifestations associated with channelopathies.

Despite the fact that Kv7.1 has traditionally been associated with cardiac function, its presence in the structures

of the central nervous system, including the brain stem and anterior parts of the CNS, as well as the occurrence of spontaneous seizures in mice with KCNQ1 mutations,[4] suggests a possible involvement of this channel in neurological processes. While these findings are preliminary, they underscore the need for further investigation into the role of Kv7.1 in neuronal hyperexcitability development.

Kv7.1 (KCNQ1) has a special place in the Kv7 family because of its tissue specificity and complex regulatory mechanisms that go beyond the classic model of potassium channels in cardiac function. Recently, there has been significant progress in understanding the molecular interactions between Kv7.1 and the β-subunit of KCNE1. This research has not only helped us to understand the kinetics of IKs current but also provided a deeper insight into the allosteric structure of the channel. It is important to note that disorders that were previously thought to be a simple loss of conductivity are often actually associated with instability in the protein complex and its dynamic behavior. This requires a revision of our pathophysiological classification systems.

What is new in our understanding of the physiology of Kv7.1 is the discovery of ion modulation of a selective filter that responds to extracellular potassium concentration by partially blocking the current, without completely closing the pore. This creates an additional layer of regulation, which depends on the ionic environment and the type of β-subunit. From a practical standpoint, this is particularly significant in pathological conditions such as ischemia, hyperkalemia, and electrolyte imbalances. These findings emphasize the importance of viewing Kv7.1 not as a static "transport protein", but rather as a dynamic entity that responds to contextual signals.

The clinical manifestations of KCNQ1 mutations are more diverse than previously believed. Various molecular mechanisms, such as intracellular trafficking disorders and instability at subunit interfaces, can give rise to similar electrocardiographic patterns. This fact challenges the assumption of universality in computational approaches and highlights the necessity for functional validation, even for mutations that seem predictable.

Of particular interest is the observation that not all mutations lead to complete loss of function. Instead, many mutations cause only a partial reduction in conductance. In this regard, the Kv7.1 channel resembles the neuronal Kv7.2/3 channel in terms of its regulation and clinical features, providing a broader framework for understanding channelopathies. The exponential growth of data regarding the extracardiac manifestation of Kv7.1, encompassing its intricate involvement in modulating insulin secretion and, potentially, exerting influence on neuronal excitability, has significantly deepened our comprehension of its multifaceted functional significance. This insight, in turn, necessitates the development of novel strategies for modeling pathological scenarios where Kv7.1 not only serves as an electrophysiological constituent but also functions as a pivotal signal integrator at the cellular level.

Despite the significant achievements made, there are still significant gaps in our understanding. The context in which Kv7.1 mutations occur in neuronal tissue is still unclear, as is the mechanism of tissue-specific expression and interaction with proteins such as calmodulin and phospholipids. Additionally, the allosteric effect of the lipid environment on the stability of the Kv7.1-KCNE1 complex remains an open question, especially under conditions of metabolic disorders.

Collectively, Kv7.1 appears to be not a simple linear channel with a limited function, but rather a complex signal-regulatory unit whose functioning is determined by a delicate balance between structural interactions, ionic environment, and context-dependent modulators. The study of Kv7.1 requires the integration of data from various fields, including structural biology, electrophysiology, systemic physiology, and clinical genetics. This makes Kv7.1 an ideal model system for interdisciplinary approaches to the study of channelopathies.

9. Kv7.2/7.3 (KCNQ2/ KCNQ3)

The KCNQ2 and KCNQ3 genes, which encode the Kv7.2 and Kv7.3 subunits of voltage-gated potassium channels, were first identified in association with benign familial neonatal seizures (BFNS) a rare hereditary disorder characterized by seizures in newborns during the first days of life, typically resolving within a few weeks.[95] These genes were discovered through positional cloning and belong to the *KCNQ* gene family, which also includes KCNQ1, encoding the cardiac Kv7.1 channel.[96]

Structural similarities particularly within the transmembrane S-domains and C-terminal regions enabled classification of these genes within the Kv7 subfamily. Functional parallels with Kv7.1 further contributed to understanding their physiological roles in the nervous system.[97,98]

The co-expression of Kv7.2 and Kv7.3 potassium channels in a single neuron gives rise to the formation of heteromeric channel complexes with heightened functional synergy. These assemblies exhibit a greater propensity for activation and display enhanced stability on the neuronal membrane when compared to their homomeric counterparts.[99–101] This intricate mechanism serves as a finely tuned regulatory system,

effectively limiting neuronal excitability by generating M-current - a slow-activating potassium current - which contributes to stabilizing the resting membrane potential and preventing repetitive depolarization events.

Mutations in the KCNQ2/3 genes are one of the leading causes of epilepsy in early childhood. The majority of these mutations have been identified in the KCNQ2 gene, affecting various domains such as the voltage sensor, gate mechanism, and C-terminus, which interacts with phosphatidylinositol-4,5-bisphosphate (PIP_2) and calmodulin.[102,103] Traditionally, it has been believed that epilepsy associated with KCNQ2 is a consequence of loss-of-function (LoF) mutations, leading to a reduction in M-currents and increased excitability, particularly during early postnatal development when inhibitory mechanisms are still developing.

Nevertheless, recent research has revealed a more intricate picture. There is increasing evidence that certain mutations may actually enhance channel activity rather than impair it. This finding has significant implications for our comprehension of how these channels operate and their role in neural function. The work of Nappi *et al.*[104] revealed that the E140R mutation stabilizes the open state of the channel, allowing it to maintain activity at membrane potentials close to the threshold. This finding suggests that this particular mutation actually enhances the functionality of the channel rather than impairing it.

Moreover, in-depth analysis using concatemers and individual channels has demonstrated that even with just one mutant subunit present, the channel remains capable of activation. This observation indicates a remarkable level of allosteric interaction within the tetrameric complex, contradicting traditional understanding of how channels function. This conundrum raises critical questions about how these mutations impact channel function and their interactions with other proteins and signalling pathways in the nervous system. Furthermore, it underscores the necessity for further research aimed at elucidating the intricate mechanisms underlying these phenomena.

Mutations in the gene KCNQ2, which codes for the Kv7.2 protein subunit, have been associated with both benign familial neonatal epilepsy (BFNE) and more severe forms such as KCNQ2-related early childhood epileptic encephalopathy (DEE) (Table 1). A clinical and genetic study conducted in China has identified 10 novel pathogenic mutations in a cohort of 23 patients.[105] The majority of these mutations were found in the S5–S6 segments and in the C-terminal domains of the protein, which are critical regions involved in the formation of the pore and electrical conduction. Apart from the typical symptoms of the disease, two novel phenotypes have been described: infantile convulsions with paroxysmal choreoathetosis (ICCA) and febrile convulsions plus (FS+). Previously, these conditions were not linked to mutations in the KCNQ2 gene.It has been discovered that sodium channel blockers, including oxcarbazepine and levetiracetam, exhibit greater efficacy in managing KCNQ2-associated DEE compared to phenobarbital. Furthermore, the precise location of the genetic mutation can serve as a predictor of the severity of the illness progression. These findings broaden the spectrum of phenotypes and genotypes linked to KCNQ2-related epileptic disorders, underscoring the importance of tailored treatment approaches based on a comprehensive understanding of the molecular mechanisms underlying the condition.

Mutations in the KCNQ2 gene continue to broaden the spectrum of epileptic channelopathies, ranging from mild forms to severe encephalopathies affecting development. Chokvithaya *et al.* have identified two novel missense variants, p.N258K and p.G279D, in infants with intractable epilepsy. These mutations cause a substantial reduction in M-current density, a shift in activation towards depolarization, and dominant negative effects when co-expressed with Kv7.3, indicating a severe loss of function and disruption of the current. This, in turn, leads to changes in passive membrane properties, including increased resistance and slowed T-response, resulting in increased neuronal hyperexcitability. This research confirms that structural abnormalities within transmembrane regions and interaction domains significantly impair M-currents in neurons, especially during the critical period of neonatal development. This knowledge should be considered when developing targeted therapies for these patients.[106] Of particular interest is the D212 mutation, which is a highly conserved amino acid residue in the S4-S5 linker. This region serves as a crucial interface between the voltage sensor and the channel gate.

A conservative amino acid substitution, such as the D212E mutation, at this position results in a substantial shift in the voltage-dependent activation of the channel and a delay in the kinetics of its opening. These modifications are associated with a more severe form of encephalopathy.[107]

In contrast, the D212G mutation leads to only a partial loss of channel function, resulting in a milder manifestation of the disease. These findings suggest that this region of the protein is highly sensitive to subtle molecular modifications.

In the context of studying KCNQ2 mutations, particular attention is focused on variants located at splicing boundaries, where even minor alterations can give rise to drastically divergent phenotypes of epilepsy. Mosca *et al.*[108] undertook a functional analysis of two such variants positioned at the

junction of intron 6 and exon 7. One of these variants, C.928-1G>C in intron, identified in an individual with self-limited epilepsy (SLFNE), disrupted splicing by eliminating ten amino acids from critical region S6 of the Kv7.2 protein (p.G310Δ10). Nonetheless, this alteration did not impact the reading frame. The other variant, C.928G>A in exon (missense G310S), associated with severe epileptic encephalopathy (DEE), likewise did not affect splicing but resulted in an amino acid replacement.

Despite the structural similarity between the two variants, both variants significantly reduced the M-current and exerted a dominant negative influence on the Kv7.2/Kv7.3 heteromeric complex. Nevertheless, there were notable discrepancies in their regulatory characteristics. The G310S mutant currents could be partially restored through the overexpression of PIP5K, an enzyme responsible for the synthesis of PI(4,5)P2 and calmodulin, indicating that the regulatory capacity of G310S remained intact. In contrast, the G310Δ10 mutant exhibited an almost complete lack of sensitivity to regulation, implying a more profound structural impairment. Moreover, there was a marked difference in sensitivity to PIP2 depletion. Complexes containing G310S appeared to be more susceptible to this lipid depletion, whereas complexes with G310Δ10 displayed a reduced dependence on it but remained functionally impaired.

These data underscore the intricate nature of the molecular processes that give rise to the clinical variability in KCNQ2-associated epilepsies, highlighting the importance of considering not only the site of the mutation, but also its influence on splicing patterns, co-factor modulation, and dominant interactions when devising treatment strategies. Thus, the integration of molecular functional analysis with comprehensive clinical characterization remains essential for implementing a personalized approach in the management of channelopathies. Of particular interest is the role of KCNQ3, a heterotetrameric partner of Kv7.2, in the molecular mechanisms underlying epileptic disorders. While most cases Sensitive S4 segment of the Kv7.3 subunit, specifically the M240R variant. Located between conserved R5 and R6 residues in S4, this variant disrupts normal channel activation of benign neonatal seizures are linked to mutations in KCNQ2, KCNQ3 has become increasingly important in clinical practice. The most intriguing variants involve the stress.

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Table 1: Overview on inherited diseases emerging from KCNQ gene mutations.

Gene/Channel	Type of Mutation (Examples)	Associated Disease	Pathophysiological Mechanism	Source
KCNQ1 (Kv7.1)	Missense (*e.g.*, R243H, G269S)	Congenital Long QT Syndrome (LQTS1), Jervell–Lange-Nielsen Syndrome	Altered voltage dependence of activation/inactivation, impaired trafficking, loss of IKs current, defective assembly with KCNE1	Sanguinetti et al., 1996[4]
KCNQ2 (Kv7.2)	Missense (*e.g.*, R213Q, A265T), nonsense, frameshift	Benign Familial Neonatal Epilepsy (BFNE), KCNQ2-related epileptic encephalopathy	Reduced M-current (IM), altered PIP2 sensitivity, impaired interaction with calmodulin, abnormal gating kinetics, subcellular mislocalization	Singh et al., 1998[95]
KCNQ3 (Kv7.3)	Missense (*e.g.*, G310V)	BFNE, KCNQ2/3-associated epilepsies	Disrupted heteromeric Kv7.2/3 function, reduced surface expression, impaired PIP2 gating, instability of channel complex	Maljevic *et al.*, 2010[96]
KCNQ4 (Kv7.4)	Missense (*e.g.*, W276S), frameshift	Non-syndromic progressive hearing loss (DFNA2A)	Impaired channel trafficking, defective VSD-pore coupling, loss of potassium conductance in cochlear hair cells	Kubisch et al., 1999 [5]
KCNQ5 (Kv7.5)	Missense (*e.g.*, Y284C, K604E)	Intellectual disability, epileptic encephalopathy, schizophrenia (rare)	Altered activation threshold, disrupted interaction with PIP2 or β-subunits, defective gating, changes in M-current amplitude and voltage-dependence, impaired excitability control	Shah et al., 2002[124] Miceli *et al.*, 2015[109]

impairment. Moreover, there was a marked difference in sensitivity to PIP2 depletion. Complexes containing G310S appeared to be more susceptible to this lipid depletion, whereas complexes with G310Δ10 displayed a reduced dependence on it but remained functionally impaired.

These data underscore the intricate nature of the molecular processes that give rise to the clinical variability in KCNQ2-associated epilepsies, highlighting the importance of considering not only the site of the mutation, but also its influence on splicing patterns, co-factor modulation, and dominant interactions when devising treatment strategies. Thus, the integration of molecular functional analysis with comprehensive clinical characterization remains essential for implementing a personalized approach in the management of channelopathies. Of particular interestis the role of KCNQ3, a heterotetrameric partner of Kv7.2, in the molecular mechanisms underlying epileptic disorders. While most cases of benign neonatal seizures are linked to mutations in KCNQ2, KCNQ3 has become increasingly important in clinical practice. The most intriguing variants involve the stress-sensitive S4 segment of the Kv7.3 subunit, specifically the M240R variant. Located between conserved R5 and R6 residues in S4, this variant disrupts normal channel activation, leading to the characteristic BFNE (benign familial neonatal epilepsy) phenotype. Electrophysiological studies reveal that this variant shifts the activation threshold towards depolarization and reduces sensitivity to changes in membrane potential. However, Kv7.2/Kv7.3 heteromers containing the M240R mutation retain residual activity, albeit with significantly reduced functional efficiency.[109].

The work of Abreo *et al.* represents a significant contribution to our comprehension of the pathogenesis of encephalopathies associated with KCNQ2.[110] Through a meticulous multidisciplinary approach encompassing molecular modeling, electrophysiological investigations, behavioral phenotyping, and subcellular localization analysis, the researchers delved into the impact of the G256W mutation within the pore domain of the KCNQ2 channel. The study uncovered the dominant negative effect exerted by this mutant subunit, attributed to its disruption of the intricate network of non-covalent interactions surrounding the S5–S6 region and selectivity filter. This disruption resulted in diminished conductivity and mislocalization of the protein away from axons. Utilizing Ezogabine, the research team partially mitigated some of these functional impairments, underscoring the intricate nature of the multifaceted mechanisms underlying this condition.

At the molecular level, alterations in voltage-gated ion channels, such as those involving mutations in the R198Q residue of KCNQ2 or the R227Q and R236C residues of KCNQ3, can disrupt the delicate balance between excitatory and inhibitory signals. This imbalance affects the activation and inactivation of these channels, altering their stability in the open state and leading to an increase in neuronal excitability, as demonstrated in recent studies.[111]

The recent findings suggest that the function of these mutant channels can be partially restored through the use of lipophilic derivatives derived from arachidonic acid. In a broader context, dysregulation of Kv7 potassium channels not only contributes to epileptic seizures but also plays a role in cortical spreading depolarization (CSD) events, which are implicated in migraine and stroke pathogenesis. Aiba and Noebels have shown that selective deletion of KCNQ2 reduces the threshold for CSD and alters the symmetrical pattern of cortical activity, thereby increasing pathological excitability.[112]

The homeostatic plasticity of M-channels manifests differently across various neuronal populations: it has been shown that their chronic modulation induces adaptive changes in excitatory, but not in inhibitory, neurons of the hippocampus, which may contribute to excitation/inhibition imbalance in pathological conditions.[113] At the same time, in fragile X syndrome, peripheral hyperexcitability of sensory neurons has been associated with impaired HCN channel function.[114]

Thus, the spectrum of genetic mutations in the KCNQ2 and KCNQ3 genes, as well as related pathophysiological processes, covers a wide range, ranging from simple structural changes to more complex dysregulation and abnormal subcellular localization of ion channels. This emphasizes the need for a multi-disciplinary approach to both research and the development of therapeutic interventions.

As a result of research conducted by Yu *et al.*, Abro *et al.*, and Mosca *et al.*, significant progress has been made in our understanding of the molecular and functional mechanisms that underlie the various forms of epilepsy. These studies have helped us to gain a deeper understanding of how different mutations, ranging from point substitutions in sensory domains to variants that affect splicing, impact the function of Kv7.2 and Kv7.3 channels, and contribute to the diversity of epileptic phenotypes.

One particularly significant achievement has been the utilization of various research techniques, such as molecular modeling, electrophysiology, subcellular analysis, and clinical phenotyping, which has not only allowed us to detect new pathogenic mutations but also to predict the severity of the condition and the effectiveness of potential therapies.

The dominant negative effects of some mutations deserve

special attention, as well as identified gain-of-function (GoF) mutations with paradoxical enhancement of channel function. This challenges the classical paradigm that loss of function is the only pathogenic mechanism. The development of new pharmacological approaches, such as activation of channels with esogabine or lipophilic derivatives of arachidonic acid, opens up promising prospects for personalized therapy. Despite these advances, several key issues remain unresolved. First, the molecular mechanisms by which Kv7 channels interact with membrane lipids, calmodulin, and other co-factors in different types of neurons and under various physiological conditions have not been fully understood. Second, predicting the clinical manifestations of these channels based solely on molecular data is challenging due to the influence of genetics, epigenetic factors, and external influences. Third, despite significant advances in pharmacotherapy, current drugs do not completely restore the normal function of mutated channels and can be accompanied by unwanted side effects.

In the future, it is essential to develop more comprehensive and integrated models that integrate molecular, cellular, and systemic levels of analysis. Additionally, we must develop selective and potent therapeutic agents that specifically target the pathophysiological mechanisms associated with each mutation class. Such an approach would enable us to more effectively translate scientific breakthroughs into clinical practice, resulting in a significant improvement in the quality of life for individuals suffering from KCNQ2/3-related epilepsy.

10. Kv7.4 (KCNQ4)

The Kv7.4 potassium channel, encoded by the KCNQ4 gene, is a critical component of the molecular machinery that stabilizes the membrane potential in specialized cells throughout various tissues. It is particularly abundant in the outer hair cells of the cochlea, where it plays a crucial role in maintaining potassium homeostasis and maintaining auditory sensitivity. Impaired potassium efflux due to Kv7.4 dysfunction results in sustained depolarization and progressive degeneration of these cells, which is the underlying mechanism of type II sensorineural hearing loss (DFNA2) (Table 1).[5,115]

However, the functional significance of Kv7.4 goes beyond the auditory system. In addition to its expression in the cochlea, Kv7.4 is also found in inner hair cells and components of the vestibular system, where it plays a stabilizing role by reducing the risk of hyperresponsiveness to mechanical stress. Studies using an acceleration-induced model in mice with KCNQ4 deficiency have confirmed the role of this channel in protecting vestibular hair cells from excessive stimulation.[116]

Kv7.4 also contributes to hyperpolarization of vascular smooth muscle cells and gastrointestinal cells, reducing their excitability and maintaining physiological tone.[85]

At the molecular level, Kv7.4 has the ability to form both homo- and heterotetrameric complexes, especially with Kv7.3. These complexes endow the resulting channels with properties similar to those of the M-type current. However, the biophysical characteristics of these complexes in vivo are influenced by their interactions with other subunits, such as KCNE4.[117] These interactions allow for precise tuning of the functional activity of Kv7.4 in a tissue-specific manner, contributing to the clinical variability observed in cases of dysfunction of this channel.

The most extensively studied pathology associated with impaired Kv7.4 function is DFNA2, a type of autosomal dominant sensorineural hearing loss. Mutations in KCNQ4 can exert their pathogenic effects through different molecular mechanisms. Dominant-negative variants, such as W276S and G285S, interfere with channel assembly by inhibiting the function of normal subunits, leading to early-onset and severe hearing loss[115]. On the other hand, haplo insufficient and deletion variants usually produce milder phenotypes characterized by late-onset and preferential high-frequency hearing loss.[118] In a landmark study, Kharkovets *et al.*[119] used mouse models with complete gene inactivation or expression of a dominant-negative KCNQ4 allele, demonstrating that both types of mutations lead to progressive hearing loss. This degeneration is accompanied by structural damage to outer hair cells due to chronic depolarization and ionic imbalance.

The traditional classification of Kv7.4 variants into loss-of-function (LOF) and dominant-negative (DOM-NET) types does not fully capture the complexity of the underlying pathogenic mechanisms. According to Homme,[120] several mutant forms of Kv7.4 induce endoplasmic reticulum stress and cytotoxicity, disrupting proteostasis and contributing to sensory cell death even when channel conduction is partially preserved. These findings open new therapeutic avenues, suggesting the use of chemical chaperones or folding correctors aimed at restoring channel expression and function.

In recent investigations, the functional analysis of variants through screening in expression systems has become increasingly important. A groundbreaking study thoroughly examined the electrophysiological properties of 4,085 single nucleotide variations (SNVs) within the KCNQ4 gene. The study identified over a thousand variants that completely disrupted the gene's function, with a substantial number exhibiting dominant-negative effects. These findings closely aligned with clinical phenotypes and data obtained from model organisms, validating the method's effectiveness and usefulness for molecular diagnostics.[121]

Additional information on the functional sensitivity of Kv7.4 to pharmacological modulation can be found in the study by Oh *et al.*[122] The authors demonstrated that a number of missense variants were partially restored under the influence of the activators retigabine and zinc pyrithione, as

well as the chemical chaperone sodium butyrate. This was especially true for the frameshift mutation p.G435Afs*61, which disrupted expression. These findings suggest the potential for pharmacological correction within the context of personalized therapy.

A similar approach was taken in another study that described a new variant of A301D in a Chinese family with isolated hearing loss. Although there was a lack of in vivo data and co-expression analysis, the electrophysiological evidence of impaired function and the strong evolutionary conservation of the corresponding amino acid residue allowed the classification of this variant as potentially pathogenic.

Kv7.4 is a versatile ion channel that plays a crucial role in the functioning of the sensory, visceral, and vestibular systems. Its malfunction not only affects the process of sound transmission but can also trigger non-conventional pathogenic processes, such as cell damage and stress responses. The combination of molecular verification, experimental models, and pharmacological studies forms the foundation for developing tailored treatments and expanding the range of diagnostic tools for suspected DFNA2.

Despite the wealth of knowledge, our comprehension of the intricate molecular and tissue-specific regulation of Kv7.4 remains incomplete. There are several aspects that require further experimental exploration, such as the interaction with other Kv7 subunits, the role of auxiliary proteins, and variations in expression along the cochlear axis. Moreover, most data on the functional consequences of Kv7 mutations were obtained in non-native systems, limiting their applicability to in vivo conditions. The impact of non-conventional mechanisms, such as endoplasmic reticulum stress and protein toxicity, on sensorineural hearing loss is also uncertain. These areas represent crucial targets for future research aimed at developing targeted molecular interventions and neuroprotective strategies for diseases associated with Kv7 dysfunction.

11. Kv7.5 (KCNQ5)

The Kv7.5 potassium channel (KCNQ5) represents the most recently identified subtype of the Kv7 family, characterized by a number of unique molecular and physiological features. Initially considered less significant than Kv7.2 and Kv7.3, it is now attracting increasing attention due to growing evidence of its role in regulating neuronal excitability and in the pathogenesis of central nervous system developmental disorders. Notably, mutations in the KCNQ5 gene have been associated with intellectual disability and early-onset epileptic encephalopathy. These findings highlight the developing brain's vulnerability to Kv7.5 dysfunction and underscore the potential importance of this channel as a therapeutic target in neurodevelopmental and neurological disorders Table 1.

Kv7.5 expression encompasses both central and peripheral components of the nervous system. It is expressed in the cerebral cortex, subcortical regions, and sympathetic ganglia, among other structures. Co-expression with Kv7.2 and K7.3 allows Kv7.5 to form heteromultimeric complexes that are involved in generating the M-current, a key mechanism for regulating neuronal excitability.[123,124] Kv7.5 can form both homotetramer and heteromer complexes with Kv7.3 and Kv7.4.[125] However, its ability to bind with Kv7.3 within the Kv7.2/Kv7.3 complex suggests that it has a fine-tuning potential that can influence the amplitude and kinetics of the M-current.

Mutations in the KCNQ5 gene are associated with a wide range of neurological conditions, from benign generalized epilepsy to severe developmental and epileptic encephalopathy (DEE), which can lead to cognitive impairment. These mutations can occur as de novo variants, which can have both loss-of-function (LOF) and gain-of-function (GOF) effects. A study by Wei *et al.*[126] found that GOF mutations, such as P369T, cause a hyperpolarization shift in the activation threshold and significantly slow down deactivation kinetics. These effects extend to interactions with other proteins, such as Kv7.3, likely through a dominant-negative interference mechanism. In contrast, nonsense variants are associated with LOF and more mild phenotypes. The severity of the V_{50} shift correlates with the severity of the clinical manifestations.

The G347S and G347A mutations in the pore domain of the Kv7.5 ion channel, which have been studied in detail in several studies, are of special interest. These amino acid changes affect a highly conserved glycine residue, leading to a disruption of the stability of the channel's closed state and an increase in the likelihood of channel opening without altering the expression level or single-channel conductance. These variants exhibit voltage-independent ionic current and a loss of sensitivity to the modulatory effect of phosphatidylinositol-4,5-bisphosphate (PIP2), indicating the existence of a novel non-phosphoinositide-dependent pathogenic mechanism distinct from classical forms of gain-of-function (GOF).[127]

At the same time, Krüger *et al.*[128] identified loss-of-function (LOF) mutations in the KCNQ5 gene in families with generalized epilepsy, including absence seizures. These missense variants, such as the R359C mutation, showed a significant reduction in the M-current, without disrupting kinetic characteristics. However, the R359C variant disrupted the interaction with phosphatidylinositol 4,5-bisphosphate (PIP_2), highlighting the importance of lipid regulation for the full function of Kv7.5. These findings suggest that some LOF mutations may be partially reversible through modulation of the phosphoinositide pathway.

It should be noted that the functional significance of Kv7.5 extends beyond the nervous system. Its presence in skel *et al* muscles and involvement in myocyte proliferation and

differentiation suggest its systemic importance in maintaining electrophysiological balance.[85] this makes Kv7.5 a potential target not only for neurological conditions, but also for muscle and possibly cardiac diseases.

In summary, Kv7.5 can be considered an independent and biologically significant regulator of ion homeostasis. It plays a role in both the stabilization of neuronal excitability and the formation of pathological conditions associated with gain-of-function (GOF) mutations. The studies conducted on this channel expand our understanding of its molecular physiology and emphasize its crucial role in the development of disorders such as drug-resistant epilepsy (DRE) and generalized epilepsy (GGE).

Despite the significant progress made in the study of Kv7.5, there are still several fundamental questions that remain unanswered, limiting our understanding of its physiological and pathological roles. The lack of a crystal structure or cryo-electron microscopy (cryo-EM) structure of Kv7.5 at atomic resolution significantly hinders our ability to predict the spatial organization of critical domains for partner binding and ligand regulation. This, in turn, limits the possibility of designing selective ligands rationally, making it difficult to fully interpret the consequences of point mutations, particularly in the pore domain and calmodulin-binding domain.

Furthermore, the mechanisms that underlie the regulation of Kv7.5 by lipids remain incompletely understood. While the functional sensitivity to phosphatidylinositol 4,5-bisphosphate (PIP_2) levels has been established, the exact location of the binding sites and their role in allosteric regulation of channel activity remain unknown. This lack of knowledge is particularly significant in light of mutations such as R359C, which disrupt lipid binding and lead to pathological phenotypes by impairing interaction with PIP_2.[128]

The final obstacle is the absence of highly specific pharmacological agents that can modulate Kv7.5 activity through gain-of-function or loss-of-function alterations. Unlike Kv7.2 and Kv7.3, for which M-activators and inhibitors are being actively developed, research on Kv7.5 is still in its early stages and is primarily limited by the extrapolation of the effects of known ligands. In conclusion, the expression pattern of Kv7.5 in the central nervous system, particularly during development and in the context of neuropathological conditions, remains largely unexplored. Given the potential for tissue-specific and age-related variations in expression, comprehending these aspects is essential for patient classification and the development of targeted therapies. The interconnected nature of these issues underscores the necessity for a comprehensive, multidisciplinary approach to studying Kv7.7, encompassing structural biology, lipidomics, cellular physiology, and neurogenetics. Only through such an integrated approach can we transition from descriptive research to a model that focuses on intervention, enabling our knowledge of Kv7.7 to translate into clinically applicable solutions.

12. Conclusion

Kv7 channels have been recognized as key components in the regulation of cellular excitability and ion homeostasis. This understanding has emerged from extensive research in the fields of electrophysiology, molecular biophysics, and clinical neurogenetics.

In addition to their role in limiting neuronal hyperexcitability, Kv7 channels act as sensitive molecular integrators, responding to various signals, including lipid interactions, calcium dynamics, redox status, and protein-protein interactions. Due to their multifaceted regulatory capacity, these channels are implicated in a wide range of physiological processes and pathological conditions such as epilepsy, cardiac arrhythmia, hearing loss, and cognitive dysfunction.

Recent advances in the field have greatly enhanced our understanding of the structure, regulation, and pharmacological modulation of Kv7 channels. Through the use of cryo-electron microscopy and new insights into lipid-binding regions, as well as the molecular mechanisms of interactions with calmodulin and other cofactors, researchers have been able to reassess the functional properties of specific isoforms, including the previously underexplored Kv7.4 and Kv7.5. This has laid the groundwork for developing selective compounds that can either activate or inhibit mutant channel variants, leading to potential new treatments for various conditions.

However, despite these advancements, several critical challenges still need to be addressed. Specifically, the mechanisms behind tissue-specific regulation need further investigation. Additionally, it is essential to understand how various mutations affect pharmacological response. Furthermore, the complex interactions between Kv7 channels and intracellular signaling remain poorly understood, hindering the full translation of basic research into clinical applications. These issues are particularly relevant under conditions of chronic inflammation, energy stress, and disruption of the membrane lipid matrix.

A transition from studying individual aspects to creating an integrated model of Kv7 channel function in vivo is becoming increasingly important. The use of advanced imaging techniques, direct measurements of biophysical properties,

and in vivo phenotyping allows the development of strategies tailored to specific isoforms and contexts. This integrated approach opens the door to precision-targeted therapies aimed at restoring Kv7 balance in various neurological and somatic conditions.

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