POTASSIUM CHANNELS AND SIGNAL TRANSDUCTION PATHWAYS IN NEURONS

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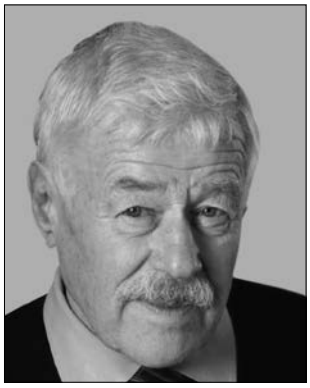

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The photos show Dr. O. Mahura and the co-workers of P.G. Kostyuk, Prof. I.S. Mahura and N.A. Bogdanova

Introduction

Potassium channels are now known in virtually all types of cell in all organisms, where they are involved in a multitude of physiological functions. K^+-channels are ubiquitous and critical for life. They are found in archaebacteria, eubacteria and eukaryotic cells, both plant and animal, and their amino acid sequences are very easy to recognize because potassium channels contain a highly conserved segment called the K^+ channel signature sequence. This sequence forms a structural element known as the selectivity filter, which prevents the passage of Na ions but allows K^+ ions to conduct across the membrane at rates approaching the diffusion limit. The K^+ selectivity filter catalyses the dehydration, transfer, and rehydration of a K^+ ion within about ten nanoseconds. This physical process is central in the production of electrical signals in biology. The selectivity filter contains two K^+ ions about 7.5 angstroms apart. This configuration promotes ion conduction by exploiting electrostatic repulsive forces to overcome attractive forces between K^+ ions and the selectivity filter. The architecture of the pore establishes the physical principles underlying selective K^+ conduction. (Doyle *et al*., 1998; Miller, 2000; Choe, 2002; MacKinnon, 2003). This is the hallmark of K^+ channels: nearly perfect selectivity for K^+ ions over Na ions in the setting of very high K conduction rates. In some members of the voltage-gated K^+ channels, removal of internal and external K^+ allows Na^+ to permeate through the pore.

K^+ channels and integration of the signals in neurons

Regulation of transcription and translation of the relevant genes exerts significant conrol over the phenotype of individual neurons. Many types of channels and receptors are expressed in the nervous system, contributing to the complex and diverse functional repertoires of neurons (Papazian, 1999). Complex processing and integration of the signals observed in neurons are facilitated by a diverse range of the gating properties of the ion channels in this cell type, particularly of the voltage-gated K^+ channels (Mahura, Zamekhovskyi, 1973; Kostyuk, 1998; Johnston et al., 2000; MacLean et al., 2003; Bezanilla, 2008). A distinctive combination the of K^+ channels endows neurons with a broad repertoire of the excitable properties and allows each neuron to respond in a specific manner to a given input at a given time. The properties of many channels can be modulated by another messenger pathways activated by neurotransmitters and other stimuli. K^+ channels are among the most frequent targets of the actions of several signaling systems.

Diversity among different members of the K^+ channel family is related mainly to the various ways in which K^+ channels are gated open. Some K^+ channels are ligand gated, which means that pore opening is energetically coupled to the binding of an ion, a small organic molecule, or even in some cases, a protein. Other K^+

channels are voltage gated, in which case opening is energetically coupled to the movement of a charged voltage sensor within the membrane electric field. Therefore, the different kinds of K^+ channels open in response to different stimuli: a change in the intracellular Ca^{2+} concentration, the level of certain G-protein subunits in the cell, or the value of the membrane voltage.

Specificity of information is generally encoded by the kinetics of action potential frequency, duration, bursting, and summation. A neuron (or specific axon or dendrite), when it is required to change its firing pattern, can rapidly regulate the gating behavior of existing channels. If longer term modifications in firing patterns are required, the cell may alter the transcriptional expression of ion channel genes for diverse functions. The number of K^+ channel genes is relatively large; however, the diversity of endogenous K^+ current phenotypes observed from various excitable cells is much greater. Additional processes such as alternative splicing, posttranslational modification, and heterologus assembly of pore-forming subunits in tetramers contribute to extend the functional diversity of the limited repertoire of K^+ channel gene products. Even greater diversity can be achieved through interactions between K^+ channel proteins and accessory proteins or subunits.

The general mechanisms of ion channel targeting are of considerable interest. Historically, K^+ channel` targeting and cellular localization were believed to involve primarily protein-protein interactions. However, there is increasing interest in the potential role of cellular lipids in the regulation of channel localization, which is the result of a revised view of membrane organization in which the traditional fluid mosaic model has been updated to reflect a developing appreciation of membrane lipid heterogeneity. The existence of membrane microdomains, particularly those referred to as lipid rafts, has motivated investigators to examine the role of protein-lipid interactions in ion channel localization more closely. Lipid rafts are specialized membrane microdomains rich in sphingolipids and cholesterol. They have been implicated in the organization of many membrane-associated signaling pathways. Biochemical and functional evidence indicate that K_v channels, in addition to other ion channels, are localized to lipid raft microdomains on the cell surface (Martens et al., 2004).

A precise control of neuronal action potential patterns underlies the basic functioning of the central and peripheral nervous systems. This control relies on the adaptability voltage gated of potassium, sodium, and calcium channel activities. The importance of voltage-gated ion channels in mediating and sculpting electrical signals in the brain is well established. Theoretical and experimental reports have explored how neurons can respond to changing inputs by adjusting their firing properties through the modification of voltage-gated ion channels (Spitzer, 1999). Recent evidence indicates that the neuronal message is persistently filtered through regulation of voltage-gated ion channels (Debanne, 2009). There are over 80 genes encoding the pore-forming subunits of the «classical» voltage — gated ion channels in mammalian neurons.

Potassium (K^+) channels are the most diverse class of ion channels, and are important for regulating neuronal excitability and signaling activity in a variety of ways. They are major determinants of membrane excitability, influencing the resting potential of membranes, wave forms and frequencies of action potentials, and thresholds of excitation. Potassium channels perform an important function in many signal transduction pathways in the nervous system. Voltage-gated K^+ channels are key components of multiple signal transduction pathways. The functional diversity of potassium channels far exceeds the considerable molecular diversity of this class of genes.

Complex processing and integration of the signals observed in neurons are facilitated by a diverse range of the gating properties of the ion channels in this cell type, particularly of the voltage-gated K^+ channels. A distinctive combination of ion channels endows neurons with a broad repertoire of the excitable properties and allows each neuron to respond in a specific manner to a given input at a given time. The properties of many K^+ channels can be modulated by the second messenger pathways activated by neurotransmitters and other stimuli.

It is now widely recognized that voltage-gated K^+ cannels exist not as independent units merely responding to changes in transmembrane potential but as macromolecular complexes able to integrate a plethora of cellular signals that fine tune channel activities. Proteins that associate with K^+ channels may act so dynamically with regulated on- and off- rates or they may be constitutively complexes for the lifetime of the channel protein. The functional result of interactions with these accessory proteins includes altered channel assembly, trafficking, protein stability, gating kinetics, conduction properties, and responses to signal transduction events (McDonald et al., 2006). Although a single type of K^+ channel ά- subunit is often present in a variety of different organs, the kinetic behavior and conformational changes of ά- subunits are often modulated by co-assembly with an ancillary subunit. The expression of ancillary subunits varies among organs, as well as among regions of an organ (Isom, 1994). This diversity contributes to the diverse assortment of potassium currents recorded from native tissues. In addition, relative expression of K^+ channels and their associated ancillary subunits can be affected by many factors such as development, changes in hormonal state, ischemic conditions, etc., which can also modulate the electrophysiology and pharmacology of native potassium currents (Bett, Rasmusson, 2008). K^+ channels encompass numerous auxiliary subunits, and many can be assembled with heteromers of multiple subunits and splice variants, rendering the combinatorial diversity of voltage-gated ion channels truly staggering (Narayanan, Johnston, 2008).

Potassium current diversity contributes to the specificity of neuronal firing patterns and may be achieved by regulated transcription, alternative RNA splicing, and posttranslational modifications. (Alternative splicing is seen in nearly all metazoan organisms as a means for producing functionally diverse polypeptides from a single gene (Black, 2000).

A single neuron can be broadly divided into three interrelated modules: *input, integration,* and *output.* Historically, voltage-gated ion channels were postulated to play a crucial role at the *output* end of a neuron. A passive integrator feeds an algebraic sum of its *inputs* to a nonlinear device (the cell body), which fires action potentials depending on the *inputs* they receive. The role of various voltage-gated ion channels in modulating the single action potentials and their bursts have been teased apart, and significant information is available about the activation, deactivation, and inactivation dynamics of various ion channels within millisecond periods. Later, equipped with the knowledge that there are conductances that are active subthreshold and that dendrites possess ion channels, the role of voltage-gated ion channels in the integration module received attention. Experimental and theoretical *evidence is* accumulating on how ion channels can contribute to integration of synaptic inputs with and without dendrites or back propagating action potentials (Narayanan, Johnston, 2008; Spruston, 2008).

Potassium channels located in the dendrites of hippocampal CA1 pyramidal neurons control the shape and amplitude of back-propagating action potentials, the amplitude of excitatory postsynaptic potentials, and dendrite excitability. Non-uniform gradients in the distribution of potassium channels in the dendrites make the dendritic electrical properties markedly different from those found in the soma (Johnston et al., 2000).

Ion channels are not only crucial in healthy individuals, but several of them have been implicated in disease, either genetic or acute. The possible treatment to channel associated disease will be accelerated if we understand in detail how the channels are implicated in the cell physiology and if we can design modifications that restore normal function (Bezanilla, 2008). For example, several human genetic diseases, such as pathologies involving cardiac arrhythmias, deafness, epilepsy, diabetes, and misregulation of blood pressure, are caused by disruption of K^+ channel genes (MacKinnon, 2003).

K^+ channels activity is modulated by external and internal K^+ ions. Elevation of $[K^+]_0$ may occur just through high levels of neuronal activity and through specific actions of neurotransmitters on glial cells. Some of the effects of changes in $[K^+]_0$ can be attributed to the shift in the K^+ equilibrium potential, which modifies both the resting cell potential and driving force for K^+ current. Variations in $[K^+]_0$ have been implicated in pathogenic of several disorders, such as *epileptyform* seizures and electrical instability of the heart following acute ischemia. These changes may occur through the modulation of K^+ channels by $[K^+]_0$ and modulating the firing pattern of neuron as a function of $[K^+]_0$ (Choe, 2002).

Two distinct molecular mechanisms for K^+-channel inactivation have been described: N-type, which involves rapid occlusion of the open channel by an intracellular tethered blocker, and slow C-type, which involves a slower change at the extra cellular mouth of the pore. The two mechanisms must be coupled in some way. Recent experiments have shown that slow C-type inactivation can be

further divided into P-type and C-type. The slow inactivation of K^+ channels can be strongly influenced by permeating ions. The cumulative inactivation of voltage-regulated K^+ channels is thought to be due to P/C-type inactivation state, from which recovery is slow (Klemic et al., 1998, 2001; Magura et al., 2004.). Cumulative inactivation appears to be state-dependent and voltage-independent. Its mechanism is similar to that in K^+ channels and is manifested in Ca^{2+} channels (Patil et al., 1998).

One of the main causes of frequency-dependent spike broadening during repetitive discharges is cumulative inactivation of certain K^+-channels. Such spike broadening can modify several aspects of neuronal signaling (Magura et al., 1971, Magura, Zamekhovsky, 1973; Aldrich, 1981; Ma, Koester, 1996).

Over many years, TEA ions have been useful probes of the structure and function of K^+ channels, perhaps because TEA is positively charged, like K^+ ions, and about the same size as a hydrated K^+ ion. External TEA blocks many types of K^+ channels but with 1000-fold range of effective concentrations (Heginbotham, MacKinnon, 1992; Tompson, Begenisich, 2003). Much of this difference can be attributed to the amino acid at a single position in the outer entrance to the pore (MacKinnon, Yellen 1990). Recent molecular dynamic simulation and electrostatic calculations suggested that the external TEA binding site in K^+ channels is outside the membrane electric field. TEA-binding site is formed by a bracelet of pore-lining aromatic residues. The bracelet center can bind a TEA through a cation-π orbital interaction.

The K^+-dependent conformational alteration which resulted in a change in $[TEA]_0$ potency was correlated with the effect of K^+ on inactivation rate. As $[K^+]_0$ was increased, $[TEA]_0$ potency and inactivation rate increased. The effects of $[K^+]_0$ on inactivation rate saturated at the same $[K^+]_0$ as the effect on $[TEA]_0$ potency. These results suggest that the different channel conformations, associated with different $[TEA]_0$ potency, can affect the rate of slow inactivation. The selectivity filter is an integral part of the inactivation mechanisms. It is the site, whose K^+ influences the channel conformation (Immke, Korn, 2000; Thompson, Begenisich, 2003).

Since K^+ channels mediate outward K^+ currents and increase the membrane conductance, they tend to hyperpolarize the cell and attenuate the effects of excitatory stimuli. They are therefore normally regarded as inhibitory, i.e. they reduce neuronal excitability. Genetic suppression of K^+ channel activity in mice causes epilepsy. Also, pharmacological blockade of K^+ channels, e.g. with 4-aminopyrine or barium**,** readily causes epileptic seizures. Compounds with K^+ channel blocking properties are commonly employed as therapeutic agents. There are a wide variety of therapeutic agents that are targeted to non-K^+ channels, but result in unintended block of K^+ channels. This K^+ channel block can result in potentially serious and sometimes even fatal side-effects (e.g. cardiac arrhythmias) (Bett, Rasmusson, 2008).

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