4 Voltage-Gated Potassium Channels

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Part I. Overview

Potassium (K^+) channels are largely responsible for shaping the electrical behavior of cell membranes. K^+ channel currents set the resting membrane potential, control action potential duration, control the rate of action potential firing, control the spread of excitation and Ca^{2+} influx, and provide active opposition to excitation. To support these varied functions, there are a large number of K^+ channel types, with a great deal of phenotypic diversity, whose properties can be modified by many different accessory proteins and biochemical modulators.

As with other ion channels, there are two components to K^+ channel operation. First, channels provide a pathway through the cell membrane that selectively allows a particular ion species (in this case, K^+) to flow with a high flux rate. Second, channels have a gating mechanism in the conduction pathway to control current flow in response to an external stimulus. To accommodate their widespread involvement in cellular physiology, K^+ channels respond to a large variety of stimuli, including changes in membrane potential, an array of intracellular biochemical ligands, temperature, and mechanical stretch. Additional phenotypic variation results from a wide range of single-channel conductances, differences in stimulus threshold, and variation in kinetics of three basic gating events. Channel opening (activation) is caused by changes in membrane potential or changes in the concentration of specific ligands, and can occur at different rates, and over different voltage or concentration ranges. Channel closing (deactivation), which occurs upon removal of the activating stimulus, can also proceed at different rates. Although the molecular events that underlie activation and deactivation are known in significant detail, the mechanisms that account for differences in activation and deactivation rates are not well understood. Finally, channels can undergo a process called inactivation, whereby the channel stops conducting even in the presence of the activating stimulus. There are at least two mechanisms of inactivation (Choi et al., 1991; Hoshi et al., 1991). One type of inactivation, which is fast (it occurs and is complete during the first several milliseconds of a strong depolarization), is called N-type because it is structurally associated with the amino (N) terminus of the channel (see Fig. 4.2). Only a few K^+ channels have an N-type inactivation mechanism. Another, slower inactivation mechanism occurs in almost all channels, and is associated with events near the selectivity filter and outer vestibule of the channel. This latter inactivation mechanism, first described in the *Shaker* K^+ channel, was originally called C-type inactivation,

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because the structural components involved were located more toward the carboxyl (C) terminus than those involved in N-type inactivation (Choi et al., 1991). However, slow inactivation has significantly different characteristics in different channels, which makes it unclear whether slow inactivation involves an identical molecular mechanism in all channels. Whereas fast inactivation is designed to rapidly turn off K^+ channel conduction following activation, slow inactivation modifies the influence of a channel depending on the frequency or duration of an excitatory event (Fig. 4.1).

4.1 Basics of K^+ Channel Structure

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An extensive discussion of the voltage-sensing mechanism is presented in Chapter 3.

The first crystal structure of a K^+ channel, KcsA, was solved in 1998 (Doyle et al., 1998). This simple K^+ channel, which consists of only two transmembrane domains, appears to provide an accurate representation of the conduction pathway of all K^+ channels. All mechanistic investigations of the K^+ channel conduction pathway are now designed and interpreted in light of structural information obtained from X-ray diffraction studies. Recently, additional, larger K^+ channel structures have been resolved by X-ray crystallography (cf. Jiang et al., 2002, 2003a,b; Kuo et al., 2003; Long et al., 2005a). Although two of these crystallized channels were voltage-gated, six transmembrane domain channels (Jiang et al., 2003a,b; Long et al., 2005a), the physical relationship between the voltage-sensing domains and conduction pathway suggested by these crystal structures has been controversial. This issue is discussed in this chapter.

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Figure 4.2 illustrates the basic structural components of K^+ channels. Each subunit of voltage-gated potassium channels has six transmembrane domains, labeled S1 through S6 (Fig. 4.2A). The S4 domain contains a repeating series of positively charged amino acid residues, mostly arginines, and is the primary domain responsible for sensing changes in voltage (Aggarwal et al., 1996; Seoh et al., 1996). Upon depolarization, the S4 domain moves, which causes a gate in the pore to open and permit ion flow. Until recently, a wide variety of evidence suggested that membrane depolarization drove the positively charged S4 domain through the membrane in an outward direction (cf. Ding and Horn, 2002, 2003; Ahern and Horn, 2004). More recent evidence, however, suggests that the actual translocation of peptide through the membrane is very minimal, perhaps only one or two angstroms (Chanda et al., 2005). The S5 and S6 domains, connected by a P (pore) loop, form the conduction pathway. The P-loop itself forms the narrow region of the conduction pathway to create the channel's selectivity filter, where selection for the passage of K^+ over the other dominant monovalent ion, Na^+, occurs. In six transmembrane domain channels, both the amino (N) and carboxy (C) termini are in the cytoplasm. A Ca^{2+}-dependent K^+ channel, BK, has a seventh transmembrane domain, S0, which puts the N-terminus on the extracellular side of the membrane. Inward rectifier K^+ channels have just two transmembrane domains, analogous to S5 and S6, with the intervening P-loop, and N and C termini in the cytoplasm.

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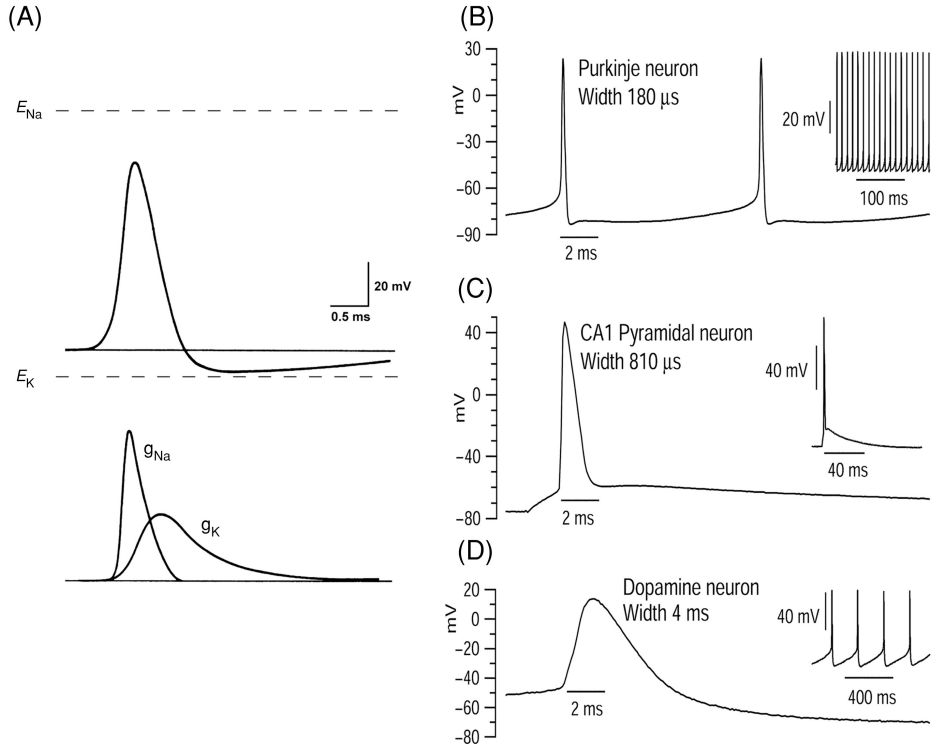

Fig. 4.1 Control of action potential duration and frequency by K^+ channels. (A) Classic illustration of the conductances that generate the squid axon action potential, adapted from Hodgkin and Huxley (1952). There are only two requirements for an action potential. The depolarizing upstroke requires the opening of voltage-gated channels (Na^+ or Ca^{2+}) to produce rapid inward currents. Repolarization requires activation of voltage-gated channels (K^+) that produce outward currents to hyperpolarize the membrane. In this simplest of action potentials, tetrodotoxin-sensitive Na^+ channels (g_{Na}) open rapidly and inactivate within approximately 0.5 ms. Delayed rectifier K^+ channels (g_K) open with a slight delay and close somewhat slowly as the membrane hyperpolarizes. Within a given class of action potentials (i.e., those carried by Na^+ or Ca^{2+}), the duration of the action potential can be essentially attributed to the time course and magnitude of the K^+ conductance. There are many different delayed rectifier channels, which, among other characteristics, open at different membrane potentials and have different rates of opening and closing. A more rapid generation of a larger g_K, via some combination of faster opening kinetics, more negative activation voltage and larger current (due to either more channels opening or larger single-channel conductance), will speed repolarization and shorten the action potential duration. Conversely, repolarization will be slower, and action potential duration longer, if g_K is smaller or more delayed. Note also that activation of gK, combined with inactivation of gNa, drives the membrane more negative to the resting membrane potential. The more quickly K^+ channels close, the sooner the membrane will be ready for another action potential, and vice versa. (B–D) Illustration of different shapes of action potentials in three types of mammalian central neurons (recorded at 35°C). These action potentials have markedly different durations, and fire with different rhythms, due to different complements of voltage-gated channels. Neurons in panels B and D fire spontaneously, but at very different rates. Neurons in panel C fire only single action potentials. (B) Action potentials from a dissociated mouse cerebellar Purkinje neuron. This neuron fired spontaneously at 87 Hz (see inset)

A complete, functional potassium channel consists of four subunits that come together to form a symmetric tetramer. [Tandem pore (2P) channels are an exception to this. These channels have either four or eight transmembrane domains, a second P-loop region, and form complete channels from dimers (Buckingham et al., 2005).] Figure 4.2B illustrates two of four subunits of the KcsA K^+ channel (Doyle et al., 1998). Each subunit consists of an outer (S5) and inner (S6) helix. The P-loop region connects the two helices, and forms the selectivity filter and outer vestibule of the channel. The KcsA pore contains four potassium ions, two in the selectivity filter, one in an inner, water filled cavity and one in the outer vestibule (Zhou et al., 2001b). The two K^+ ions in the selectivity filter are separated by a water molecule, and can occupy two of four locations, positions 1 and 3 (open circles) or positions 2 and 4 (shaded circles). The inner helices (S6 domain) form a bundle crossing at the cytoplasmic entrance to the conduction pathway in KcsA, but are probably arranged in a slightly different orientation in Kv channels (del Camino et al., 2000). This bundle crossing is the general location of the cytoplasmic activation gate, whose opening is linked to the S4 domain movement in voltage-gated channels.

Figure 4.2C illustrates the N- and C-terminal domains in more detail (again, just two subunits are shown). The N-terminal domain of voltage-gated channels contains a tetramerization domain (T1 domain; ribbon), which comes together just underneath the conduction pathway, in a fourfold arrangement resembling a "hanging gondola" (Kobertz et al., 2000). Several, but not all, voltage-gated channels also have a globular, positively charged structure, the N inactivation ball, at the end of the N-terminus. When channels are opened by depolarization, one of the four inactivation balls rapidly enters the conduction pathway and blocks it, leading to N-type inactivation (I_N; Fig. 4.1D). The C-terminal domain, illustrated as a shaded oblong structure, varies considerably in size in different K^+ channels, associates with both the cytoplasmic end of the conduction pathway and the N-terminal domain, and is an important modulator of K^+ channel function.

Figure 4.2D illustrates in cartoon form the four basic gating states of a K^+ channel. Panel 1 illustrates a channel with a closed cytoplasmic gate (note that four potassium ions are illustrated to be in the pore here, but the number of K^+ ions in this

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Fig. 4.1 (*continued*) in the absence of current injection, as is typical of Purkinje neurons. Kv3 channels, which open and close very quickly, contribute the dominant delayed rectifier current. The rapid opening and closing kinetics of Kv3 channels allow for very high-frequency firing of action potentials. Note that the action potential is so brief in panel B that Na^+ channels undergo little or no inactivation. Recording by Andrew Swensen. (C) Action potentials in rat CA1 pyramidal neuron recorded from a hippocampal brain slice (stimulated by 2 ms injection of current to reach threshold). Kv1, Kv4 and possibly some Kv2 or Kv3 channels all contribute to action potential repolarization. Recording by Alexia E. Metz. (D) Action potentials in mouse midbrain dopamine neuron, recorded from a brain slice. This cell fired with a typical rhythmic pacemaking activity at ~4 Hz. The K^+ channels responsible for repolarization have not yet been identified. Recording by Michelino Puopolo. Fig. B–D was kindly provided by Bruce Bean and Marco Martina. Details can be found in Martina et al. (1998), Mitterdorfer and Bean (2002), Martina et al. (2003), Puopolo et al. (2005, and references within).

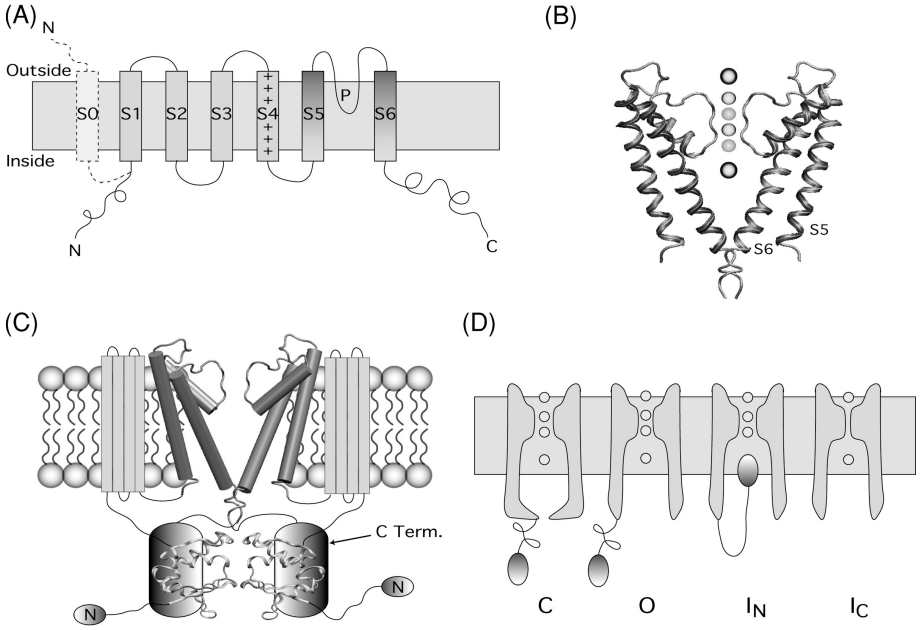

Fig. 4.2 Basics of K^+ channel structure and states. (A) Cartoon of the fundamental K^+ channel subunit. (B) Channels are formed from four subunits arranged symmetrically around a central core. In this panel, two opposing subunits are shown; the front and rear subunits were omitted for illustration clarity. The selectivity filter is formed between the S5–S6 pairs of transmembrane domains. The locations of K^+ binding sites in the conduction pathway denoted by spheres. (C) Schematic of two subunits of a Kv channel, showing N-terminal domain (ribbon) and C-terminal domain (oblong blocks) underneath the pore. In some channels, the distal portion of the N-terminal domain contains a charged, yet hydrophobic peptide sequence (the inactivation particle N). Upon depolarization, the charged peptide is driven into the pore with a delay, causing N-type inactivation (see panel D). (D) Illustration of four channel states, closed (C), open (O), N-type inactivated (I_N), and C-type inactivated (I_C).

and other channel states is currently unknown, and may in fact differ in different K^+ channels). Upon depolarization, the cytoplasmic gate opens (panel 2). In channels with an N inactivation ball, open channels quickly become blocked by the inactivation ball (panel 3), creating a transient current. Almost all K^+ channels, whether they have an N inactivation ball or not, undergo a second inactivation mechanism, which apparently involves a constriction at or near the selectivity filter (panel D).

4.2 Functional Classification

There are over 150 K^+ channel species, which can be broadly characterized into several main types: voltage-gated (Kv), Ca^{2+}-dependent (K_{Ca}), inward rectifier (Kir), and tandem pore (2P). Fully functional channels, formed by primary (α) subunits, are often associated with accessory (β) subunits. The β subunits also come in different

flavors, some of which are apparently restricted to the cytoplasm (e.g., β1, β2, KChIPs) and some of which are small membrane spanning subunits (MinK, MiRP). β subunits, each of which can interact with a host of different α subunits, modify α subunit characteristics such as ion selectivity, gating kinetics, surface expression, and pharmacology. In addition, there are also silent α subunits, which do not form functional channels by themselves. They can, however, combine with functional α subunits to form heterotetramers with somewhat altered properties (Kramer et al., 1998; Shepard and Rae, 1999; Ottschytsch et al., 2002; Kerschensteiner et al., 2003). Relatively little is known about the role of these silent α subunits in cell physiology.

4.2.1 Voltage-Gated Channels

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There are four major families of Kv channels, Kv1.x to Kv4.x, currently with 2–8 members in each family (Gutman et al., 2003). The first cloned potassium channel was called Shaker, after the mutant fruit fly from which it was identified (Papazian et al., 1987). This channel is equivalent in the Kv nomenclature to Kv1.0, and due to its extensive study early on, retained its name and became the prototypical Kv channel.

Kv channels are generally activated by depolarization, and primarily serve to control the duration, shape and firing frequency of action potentials. Thus, rapidly activating, slowly inactivating Kv channels keep action potentials brief (∼2 ms), whereas slowly activating Kv channels allow for more prolonged action potentials, (for example, cardiac myocytes have action potentials >200 ms in duration, partially as a result of a very slowly activating Kv channel). Whereas there is certainly overlap of function, different voltage-gated channels are specialized for certain functions. For example, in contrast to most other K^+ channels, current through the Kv2.1 channel increases as external K^+ concentration ($[K^+]$) is elevated (Wood and Korn, 2000). This unusual feature apparently predisposes the Kv2.1 channel to be of special importance during high-frequency firing (Du et al., 2000; Malin and Nerbonne, 2002), when external $[K^+]$ tends to rise and other Kv channel currents diminish due to the consequent reduction in electrochemical driving force. The HERG potassium channel, which is also activated by depolarization, has the unusual property of inactivating faster than it activates with strong depolarizations (such as produced by an action potential). Upon membrane repolarization, channels exit from the inactivated state more quickly than they close. Consequently, current through these channels remains low until after repolarization begins. During repolarization, as current through other open K^+ channels diminishes, current through HERG channels increases, thus adding to the speed of repolarization. The Kv3.x channels are generally localized where information is encoded by the high-frequency firing of action potentials (Rudy et al., 1999). These channels activate rapidly, which hastens repolarization and limits Na^+ channel inactivation. They also deactivate extremely rapidly, which thus shuts off hyperpolarizing current. These two properties keep action potentials brief and the membrane poised to immediately fire a subsequent action potential. Finally, some members of the Kv1, Kv3, and Kv4 channel families also have a rapid inactivation

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mechanism. Rapidly inactivating channels have several functions, which include regulation of the frequency of rhythmic action potential firing (Connor and Stevens, 1971a,b) and integration of electrical activity in neuronal dendrites (see Part III).

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4.2.2 Ca^{2+}-Dependent Channels

In excitable cells, K_{Ca} channels come in two varieties: large conductance (maxi-K or BK) and small conductance (SK). BK channels are also activated by depolarization, but channel activation is greatly facilitated by elevation of intracellular Ca^{2+}, which can result either from depolarization-evoked entry of Ca^{2+} through Ca^{2+}-permeable channels or from release of Ca^{2+} from intracellular stores. As a result of this Ca^{2+} dependence, BK channels produce a long, post-action potential hyperpolarization, which keeps the membrane potential away from firing threshold and thus delays the membrane's ability to undergo another action potential. In contrast, SK channels are activated solely by elevation of intracellular Ca^{2+} (Bond et al., 1999). The strict dependence on intracellular Ca^{2+} means that their influence increases as intracellular Ca^{2+} builds up, such as during high-frequency firing of action potentials. Thus, they may serve primarily to terminate long bursts of action potentials (Swensen and Bean, 2003).

4.2.3 Inward Rectifier Channels

As a generalization, Kir channels help maintain the negative resting potential. They are open at negative membrane potentials, and consequently oppose excitation by small excitatory stimuli. Upon significant depolarization, they are blocked by intracellular small molecules (Mg^{2+} or polyamines) so as not to be a continuing factor during the excitation event. Kir channels can be modulated by G-proteins as well as other intracellular biochemicals, which demonstrates that modulation of their ability to suppress cell excitation is an important functional feature of these channels. One highly complex Kir channel, KATP, is sensitive not only to the intracellular molecules and modulators that influence other Kir channels but also to intracellular ATP. Elevation of intracellular ATP inhibits channel activity, which links the influence of these channels to changes in cell activity and metabolism (cf. Tarasov et al., 2004).

4.2.4 2P Channels

Tandem pore (2P) K^+ channels have two P-loop regions and come in two varieties: one member isolated from yeast (TOK1) has eight transmembrane domains, whereas all other known members have four (Goldstein et al., 2001; Kim, 2003; Buckingham et al., 2005). The role of 2P channels, discovered fairly recently in the overall history of ion channel research, is still unclear. Initially, they were thought to be constitutively open (Goldstein et al., 1996) and thus came to be called, ingloriously, "leak" channels. For decades, the basis of the resting membrane conductance to K^+ remained a mystery. These channels appear to represent the solution to the mystery. In addition,

recent work has shown that opening of 2P channels can be regulated by fatty acids, temperature, intracellular pH and membrane stretch (Lopes et al., 2000; Kim, 2003; Kang et al., 2005). Consequently, these channels may not only contribute to the steady resting conductance, but may also turn out to be actively involved in cellular function (cf. Richter et al., 2004).

4.3 Summary

There are more than 150 known potassium channel genes (Goldstein et al., 2001; Gutman et al., 2003), and there can be many K^+ channel species in any given cell membrane. The general role of each channel phenotype in membrane electrophysiology is reasonably well understood. Consequently, much of the effort toward understanding function is now focused on (1) the myriad biochemical mechanisms by which each channel function is modulated and (2) how each of the many channels in a particular cell type specifically influences cell function. In the last 15 years, the maturation of molecular biological approaches has led to an extensive understanding of the structural basis of ion channel function. The structures underlying the voltage-sensor and the fast inactivation gate were found (Aggarwal and MacKinnon, 1996; Seoh et al., 1996; Aldrich, 2001), the structure underlying the selectivity filter was found (Heginbotham et al., 1994; Doyle et al., 1998; Zhou et al., 2001b), and the general mechanism of conduction through multi-ion pores has been reasonably well described. The more recent ability to obtain structural information from X-ray crystallography, and the use of NMR spectroscopy with knowledge of these structures, has once again accelerated the pace of discovery. Knowledge of the structure itself has constrained and suggested mechanism. With knowledge of the fundamental pore structure, molecular studies of gating mechanisms and permeation properties can now be designed and interpreted more astutely. Recently, description of the structures of voltage-gated and Ca^{2+}-dependent channels has led to a more sophisticated understanding of gating. Indeed, perhaps the most invigorating recent publication in the field of ion channel biophysics was the crystallization of a voltage-sensitive K^+ channel (Jiang et al., 2003a,b). The proposed structure of the voltage sensor was at odds with years of biophysical measurements, and the push to reconcile the two led to the rapid design of critical experiments that greatly accelerated our understanding of gating mechanisms (Ahern and Horn, 2004). A more recent crystal structure of another voltage-gated channel (Long et al., 2005a) presents a structural picture that more closely matches results from biophysical experiments, yet incompatibilities between the structural and biophysical data remain. Resolution of these incompatibilities will surely refine our understanding of the ion channel mechanisms. Finally, it is clear that over the last 25 years, most biophysical measurements have been made either on naked α subunits, in the absence of potential modulators, or on α ubunits that are complexed with unknown stoichiometry to unknown modulators. The advance of molecular and genetic technology has led to the discovery of an enormous number of these modulators, and with continuing sophistication in molecular, structural,

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biophysical, and theoretical approaches, a new surge in mechanistic discovery is imminent.

Part II. K^+ Channel Operation

4.4 Control of Single-Channel Conductance

The primary function of an ion channel is to permit the rapid flux of ions across a cell membrane. The potassium channel family displays a large range of single-channel conductances, ranging from 2 to 240 pS. This range certainly reflects the diversity of functions required of K^+ channels. How single-channel conductance is varied so greatly among structurally similar channels is one of the great fundamental questions remaining about ion channel function. Indeed, it is not known whether there is a general structural basis for control of conductance or whether conductance is limited at different locations in different channels.

4.4.1 Role of the Selectivity Filter

One school of thought has been that the selectivity filter would be rate limiting for conductance. This possibility is derived from the fact that the filter is the narrowest, and presumably the most electrically resistive, region of the pore. Furthermore, to enable high selectivity for one ion over a very similar ion, one might infer that the more permeant ion also has a relatively high affinity for the selectivity filter. There is some evidence that subtle variations in the selectivity filter might influence single-channel conductance. For example, mutations can be made to residues near or within the selectivity filter that change single-channel conductance without destroying ion selectivity (Zheng and Sigworth, 1997; Lu et al., 2001; So et al., 2001). However, there are two compelling arguments against a primary role of the selectivity filter in determining single-channel conductance. First, the structure of the K^+ channel selectivity filter is highly conserved among members of the K^+ channel family that have widely varying single-channel conductances. This structural similarity makes the selectivity filter an unlikely location for significant functional variation. Second, it has been suggested, based on the structural data from the KcsA channel, that the selectivity filter is always fully occupied by two K^+ ions, and is designed for very high throughput (Morais-Cabral et al., 2001; Zhou et al., 2001b; Zhou and MacKinnon, 2003, 2004). Interestingly, the conductance of the KcsA channel increases linearly up to very high [K^+], which suggests that the filter presents almost no barrier to conduction at physiological [K^+]. Theoretical simulations, based on the structural data, suggest that ions in the selectivity filter of conducting channels are in a stable equilibrium, with little energy needed to push ions through the filter (Bernèche and Roux, 2001; Chung et al., 2002). Whether this is true for all potassium channels remains to be determined. For example, biophysical studies suggest that the

selectivity filter in the Kv2.1 channel is not fully occupied in conducting channels (Immke and Korn, 2000).

The interplay between experimental studies and sophisticated simulation models has already produced, and will continue to produce, a plethora of insight into ion conductance through the selectivity filter. For example, in some channels, conduction of K^+ and the very similar Rb^+ can be markedly different. The ability to (1) determine differences in occupancy of the selectivity filter by K^+ and Rb^+ (cf. Zhou and MacKinnon, 2003), (2) manipulate the molecular structure of the selectivity filter, and (3) make rigorously consistent models that account for both the structural and biophysical data, will undoubtedly yield a greater understanding of how the selectivity filter accommodates high flux while maintaining high selectivity, as well as potentially explaining differences in conduction among different K^+ channels (cf. Zhou and MacKinnon, 2004).

4.4.2 Role of the Inner Vestibule

Recently, experimental and theoretical studies have suggested that ion entry into the inner vestibule may be a key determinant of conductance magnitude. The BK channel, so named because of its "big" conductance, has a ring of glutamates near the inner entryway of the channel (Brelidze et al., 2003). Removal of these glutamates significantly reduces single-channel conductance (Brelidze et al., 2003). Conversely, insertion of glutamates into KcsA in this same region significantly increases conductance, although not to the level observed for BK (Nimigean et al., 2003). These studies suggest that the ring of negative charges lowers the energy barrier for ion entry into the pore and thus facilitates conduction. Interestingly, studies of internal quaternary ammonium (QA) block suggest that BK channels have a larger inner vestibule than other K^+ channels (Li and Aldrich, 2004). This is consistent with theoretical studies, which suggest that enlargement of the inner vestibule can dramatically increase single-channel conductance (Chung et al., 2002).

4.4.3 Role of the Outer Vestibule

In some channels, K^+ exit from the selectivity filter into the outer vestibule may also be rate limiting for conductance. An early study demonstrated that a chimeric channel, in which the outer vestibule region of the Kv2.1 channel was replaced by the equivalent region of the Kv3.1 channel, displayed the conductance characteristics of the channel that contributed the outer vestibule region (Hartmann et al., 1991). More recently, it was shown that a positively charged lysine in the outer vestibule of Kv2.1 reduces single-channel conductance by interfering with K^+ exit from the selectivity filter (Consiglio et al., 2003; Trapani and Korn, 2003). Moreover, physiologically relevant conformational changes in the outer vestibule, which reorients this lysine, alters single-channel conductance (Trapani et al., ~~2005~~). Thus, it may be that there are multiple locations used by different channels to limit single-channel conductance.

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4.5 Activation Gates

Permeation through ion channels is gated, and clearly, understanding the control of current flow through a channel requires a comprehensive understanding of gating mechanisms. At a minimum, there needs to be one gate that opens and closes on command to start and stop flux through the pore. However, recent data suggest that there may be a second activation gate.

4.5.1 The Cytoplasmic Gate

Upon depolarization of the membrane, the voltage sensor moves, the gate inside the pore opens and current flows. In a series of papers published in the early 1970s, Armstrong and Hille presented evidence which suggested that the activation gate in the squid K^+ channel was located toward the intracellular end of the channel entrance (Armstrong and Hille, 1972). Briefly, these studies showed that quaternary ammonium (QA) ions in the intracellular solution gained access to their blocking site after the channel opened, and that they appeared to be trapped within the channel upon channel closing (Armstrong, 1971; Armstrong and Hille, 1972). Subsequent molecular (Choi et al., 1993) and structural (Zhou et al., 2001a) studies demonstrated that internal QA ions bind at the internal entrance to the selectivity filter, deep within a large aqueous central cavity. Furthermore, cysteine accessibility studies demonstrated that even small cations (e.g., Ag^+) could not pass through the activation gate and enter the central cavity region when channels were closed (del Camino and Yellen, 2001; but see Soler-Llavina et al., 2003 for a quantitative description of how closed a closed channel is). These studies confirmed Armstrong's essential conclusion that a voltage-sensitive gate formed a trap door at the intracellular entrance to the pore.

The location of the cytoplasmic gate was refined by cysteine scanning studies (Liu et al., 1997). Single-cysteine substitutions were made along the S6 domain of the *Shaker* channel and a very simple question asked: which cysteines could be modified by applied thiol-reactive chemicals when the channels were closed, and which cysteines required the channel to be open? Using this approach, Liu et al. (1997) demonstrated that several residues located toward the selectivity filter were relatively inaccessible when channels were closed but became accessible upon channel opening. In contrast, a cysteine located five residues closer to the cytoplasmic entrance of the channel was exposed similarly in both closed and open channels. These results placed the cytoplasmic gate at a defined region at the cytoplasmic entrance to the pore. The gate prevented entry of both positively and negatively charged cysteine modifying reagents, which indicated that gate closing was based on steric, not electrostatic, occlusion (del Camino and Yellen, 2001). Subsequently, a completely different approach, which evaluated mutation-induced disruption of gating, also placed the intracellular gate at this same region of the pore (Hackos et al., 2002).

4.5.2 Coupling of the Voltage-Sensor to the Cytoplasmic Gate

It remains to be conclusively determined how the voltage-sensor is coupled to the cytoplasmic gate, and how movement of the voltage-sensor causes the gate to open and close. Some evidence, however, is beginning to emerge. Lu et al. (2001, 2002) made chimeras between *Shaker*, a voltage-gated channel and KcsA, a non-voltage-gated channel. Remarkably, concatenating the voltage-sensing domains from *Shaker* to the two transmembrane domain channel, KcsA, provided the ability to gate KcsA by voltage. Lu et al. (2002) found two regions near the cytoplasmic end of the channel that were essential for coupling of the voltage-sensor to the cytoplasmic gate: the S4–S5 linker region and a critical sequence near the carboxy-terminal end of S6. This latter region corresponds to the location of the cytoplasmic gate defined by studies described above. Studies of this interaction in the HERG potassium channel and the HCN channel (a related, hyperpolarization-activated channel) suggested that movement of the voltage-sensor disrupts a specific, perhaps electrostatic, interaction between the S4–S5 linker and the C terminus near the carboxy-terminal end of S6 (Tristani-Firouzi et al., 2002; Decher et al., 2004). A recently crystallized voltage-sensitive channel provided new structural information regarding the physical association of the S4 domain with the pore (Long et al., 2005b). From these structural data, the authors presented a model as to how movement of the voltage sensor might perform mechanical work on the pore, via the S4–S5 linker, to open a gate.

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The Selectivity Filter may be an Activation Gate

4.5.3 ~~Is the Selectivity Filter also an Activation Gate?~~

Most potassium channels undergo a slow inactivation process, whereby current is curtailed despite a stimulus for channels to be open. Extensive experimental investigation has demonstrated that slow inactivation involves a cut off of ion flux at the selectivity filter (see below). It is thus widely accepted that the selectivity filter can act as an inactivation gate. Although inactivation may be coupled to activation, these two processes can be independently manipulated, and almost certainly represent two independent mechanisms. Whereas there is no direct evidence for a role of the selectivity filter in channel activation in voltage-gated channels, a great deal of circumstantial evidence suggests that it may indeed be an additional activation gate.

4.5.3.1 Voltage-Independent Gating Transitions

Most K^+ channels display rapid close–open transitions, even when fully activated. Bao et al. (1999) studied the kinetics of these transitions in a Kv channel (*Shaker*) that had its voltage-sensor disabled. This channel, which was constitutively activated, displayed rapid close–open transitions with essentially identical kinetics to that observed in the open, wild-type, voltage-sensitive channel. These data were consistent with the presence of a gate whose operation was unaffected when the pore was functionally uncoupled from the voltage sensor. Although one could argue that this gate

was the cytoplasmic gate, it seems unlikely that a single structural gate would flicker identically whether coupled to a crippled S4 domain or a functional S4 domain in a fully activated channel.

4.5.3.2 Mutagenesis Near the Selectivity Filter

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Liu and Joho (1998) demonstrated that a mutation in the S6 transmembrane domain of the Kv2.1 voltage-gated channel, near the selectivity filter, altered the voltage-independent, rapid gating transitions. In addition, mutation of different amino acids at this location altered single-channel conductance selectively for different ions. These authors postulated that this mutation, located a significant distance from the cytoplasmic gate, influenced the operation of a second gate, which they considered to be the selectivity filter. Their two-gate hypothesis was strengthened by the subsequent demonstration that a different mutation, thought to be within the conduction pathway, influenced the voltage-dependent gating mechanism but not the voltage-independent gating transitions observed with the first mutation (Espinosa et al., 2001).

Experiments that utilized mutagenesis within the selectivity filter of both inward rectifier and Kv channels also supported a model of the selectivity filter as an activation gate. Inward rectifier potassium channels are not gated by voltage but rather, by a channel block mechanism (Lu, 2004). Thus, outward current is prevented upon depolarization due to block by intracellular Mg^{2+} or polyamines. In the absence of blocker, however, single-channel records demonstrate clear open–close transitions. Mutations in the selectivity filter of the Kir2.1 inward rectifier altered the mean open time of the channel, as well as transitions between conductance states (Lu et al., 2001; So et al., 2001). Similarly, in *Shaker*, mutation of the residue that forms the innermost part of the selectivity filter increased channel open time even when channels were fully activated by depolarization (Zheng and Sigworth, 1997). Moreover, heteromeric channels, which contained different numbers of mutations at this selectivity filter location, displayed different conductance sublevels and, at each conductance level, different ion selectivity (Zheng and Sigworth, 1998). These results suggested that the transitions among conductance sublevels in fully activated channels were associated with the operation of the selectivity filter. However, other than the vague postulate that conformational changes account for this flicker, a mechanistic description of how these gating events might occur at the selectivity filter has remained elusive (but see Bernèche and Roux, 2005, for an interesting hypothesis).

4.5.3.3 Accessibility Studies

Proks et al. (2003) asked whether intracellular Ba^{2+} block of the ATP-sensitive inward rectifier, Kir6.2, was altered by closing of the activation gate. ATP binding stabilizes Kir6.2 channels in the closed conformation via an allosteric mechanism. Thus, in the presence of ATP, transitions of Kir6.2 into the closed state are more frequent and prolonged. Ba^{2+} block occurs at the inner end of the selectivity filter (Neyton and Miller, 1988; Jiang and MacKinnon, 2000). Proks et al. (2003) predicted that the rate and apparent affinity of internal Ba^{2+} block would be reduced in the

presence of ATP if the gate were closer to the cytoplasmic entrance of the pore than the Ba^{2+} blocking site. In contrast to this prediction, closing of the gate with ATP slightly increased the rate of Ba^{2+} block, and produced little or no change in apparent Ba^{2+} affinity (Proks et al., 2003). These results put the ATP-activated gate above the Ba^{2+} block site, and thus at the selectivity filter.

As described above, differences in cysteine accessibility in open and closed channels can be used to define the location of a gate. This approach was also used to search for the location of the gate in SK channels and cyclic nucleotide-gated (CNG) channels. These two channel types contain six transmembrane domains, and are structurally similar to Kv channels. However, neither is activated by a change in membrane potential. SK channels are K^+-selective channels activated by intracellular Ca^{2+}, CNG channels are nonselective cation channels activated by intracellular cyclic nucleotides. Bruening-Wright et al. (2002) demonstrated that the cysteine-modifying reagent, MTSEA, had equal access to a cysteine in the inner vestibule of open and closed SK channels. This residue is located between the presumed location of the cytoplasmic gate and the selectivity filter. Similarly, Flynn and Zagotta (2001) demonstrated that Ag^+ could access a cysteine at an approximately equivalent location equally well in closed and open CNG channels. Based on the structural information that there isn't a constriction in the pore between the location of a possible cytoplasmic gate and the selectivity filter, these data suggested that the selectivity filter was the activation gate in these two channels. Interestingly, the larger cysteine-modifying reagent, MTSET, had differential access to this inner region of the pore in open and closed channels (Flynn and Zagotta, 2001; Bruening-Wright et al., 2002). The potential significance of this result will be discussed below.

4.5.4 Coupling Between the Cytoplasmic Gate and Selectivity Filter Gate

If there are two activation gates, are they energetically (or mechanistically) coupled? Several studies suggest that they are. First, the subconductance state experiments by Zheng and Sigworth (1997), described above, suggested that the two activation mechanisms were energetically coupled. Second, NMR studies of KcsA indicated that during gating, both the cytoplasmic gate region and the region adjacent to the selectivity filter change conformations (Perozo et al., 1999). Third, an extensive mutant cycle analysis performed on the *Shaker* pore led to two findings: that residues near the cytoplasmic gate and near the selectivity filter are coupled to activation and that residues in these two regions are energetically coupled to each other (Yifrach and MacKinnon, 2002).

The accessibility studies described above present an intriguing possibility about coupling between the gates. In both SK and CNG channels, even though relatively small cysteine-modifying reagents entered the pore equally in closed and open channels, the larger MTSET had differential access to the inner pore in closed and open channels (Flynn and Zagotta, 2001; Bruening-Wright et al., 2002). These results

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demonstrate that, during activation, the region typically associated with the cytoplasmic gate still undergoes a conformational change, but that it doesn't form a barrier to ion conduction in the closed state. Thus, the conformational changes associated with gating in channels that use either the cytoplasmic gate or the selectivity filter gate may not be all that different. Indeed, it may be that in voltage-gated channels, the cytoplasmic gate fully closes and the rapid conformational changes at the selectivity filter, perhaps controlled, perhaps not, underlie the rapid, close–open transitions. In contrast, channels that are gated by intracellular ligands (e.g., SK, CNG, KATP) may have an open cytoplasmic end of the pore at all membrane potentials. However, ligand-induced conformational changes at the cytoplasmic end of the pore (the location of the voltage-sensitive gate in Kv channels) may trigger gating at the selectivity filter. Thus, the fundamental mechanism of gating may be conserved among all of these different types of channels, and differences in gating mechanisms may then be seen to reflect variations on the basic theme.

4.5.5 Why Have Two Gates in One Channel?

The magnitude of current flow through a single channel is determined by two factors: single-channel conductance and mean open time. With a single gate, current magnitude in a fully activated channel will be determined by the single-channel conductance alone. Adding a second gate provides yet additional diversity to channel operation. Once the inner gate is open, the mean open time will be determined by fast open–close transitions that apparently occur at the selectivity filter. Whereas the cytoplasmic gate opening appears to respond primarily to voltage, gating at the selectivity filter may provide a locus for voltage-independent modulation of gating.

4.6 Functions of the Outer Vestibule

The outer vestibule is relatively small compared to the rest of the potassium channel (Doyle et al., 1998). Nonetheless, it is the site of many important channel functions. In addition to its role in regulating conductance in at least one channel (described above), it is the location for block by many naturally occurring toxins and the prototypical small molecule open channel blocker, tetraethylammonium (TEA), and is involved in the process of slow inactivation. Recent evidence suggests, however, that we may understand less about these latter two events than we think.

4.6.1 The TEA Binding Site

TEA is a cationic K^+ channel pore blocker that acts at an internal binding site when applied from the cytoplasm and at a separate, external binding site when applied from the extracellular solution. It has been used for decades as a tool to investigate K^+ channel pharmacology, permeation, gating, structure, and dynamics of protein movement. TEA inhibits most potassium channels. This lack of selectivity in the

outer vestibule has been advantageous, in that much has been learned about the outer vestibule as a result of the variation in TEA potency, which ranges from low mM to >100 mM, among K^+ channels. Mutagenesis studies in the early 1990s demonstrated that the amino acid residue at *Shaker* position 449, believed to be just external to the selectivity filter, had a dramatic impact on TEA potency (MacKinnon and Yellen, 1990; Heginbotham and MacKinnon, 1992). Indeed, channels that are not blocked by TEA typically (but not always) have a positively charged residue at the equivalent position and neutralization of this residue can restore TEA block in an otherwise TEA-insensitive channel. The greatest TEA potency occurs in channels with an aromatic residue at this position (Heginbotham and MacKinnon, 1992). Studies of heterotetramers demonstrated that all four position 449 residues contributed approximately equally to TEA potency (Heginbotham and MacKinnon, 1992; Kavanaugh et al., 1992). These studies led to a model in which TEA was coordinated by the side chains at position 449. Indeed, the position equivalent to 449 in *Shaker* became known as "the TEA binding site."

Molecular dynamics studies that modeled TEA binding using the KcsA channel structural information came to much the same conclusion (Crouzy et al., 2001). In these models, the energy minimum for TEA in the outer vestibule was located very near the equivalent of position 449 residues when aromatic residues (native to KcsA) were placed at this site. With threonine (native to Shaker) at this site, the TEA molecule was less dehydrated and located in a somewhat more external position.

4.6.2 Slow Inactivation

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Fluorescence measurements demonstrated that a residue in the outermost region of the outer vestibule moves during inactivation (Loots and Isacoff, 1998), and mutation of this same externally located residue alters inactivation kinetics (Jerng and Gilly, 2002; Kehl et al., 2002). The volume of the inner vestibule, and thus inner vestibule conformation, also appears to change during slow inactivation (Jiang et al., 2003a~~,b~~), which suggests that inactivation involves a widespread structural reorientation. Nonetheless, there is general agreement that closing of the inactivation gate involves a constriction of the pore just external to, and possibly at, the selectivity filter. This picture is derived in large part from four observations, primarily made using the *Shaker* potassium channel as a model system. First, during slow inactivation in *Shaker* (which displays the prototypical slow inactivation mechanism called "C-type" inactivation), the residues at position 449 move relative to their surroundings (Yellen et al., 1994). If cysteines are introduced into this position, the affinity of Cd^{2+} for these cysteines increases ~45,000-fold during inactivation (Yellen et al., 1994). This observation led to a model which postulated that, as the constriction progressed during inactivation, the cysteine side chains at position 449 on each subunit moved closer together, so that an increased number of side chains contributed to the coordination of a single Cd^{2+} (Yellen et al., 1994). Second, and perhaps most persuasive, cysteines at position 448, one residue deeper into the pore, crosslink during inactivation (Liu et al., 1996). Thus, the cysteine side chains at position 448 must

move closer together as channels inactivate. Third, occupancy of the selectivity filter by K^+ slows classical C-type inactivation (Baukrowitz and Yellen, 1996; Kiss and Korn, 1998), which suggests that the constriction could not occur with a K^+ in the selectivity filter. Finally, the ability to conduct the smaller Na^+ ion increases during the inactivation process (Starkus et al., 1997; Kiss et al., 1999; Wang et al., 2000), which is again consistent with the diameter of the selectivity filter decreasing during inactivation.

4.6.3 Model of TEA Binding and Slow Inactivation

External TEA slows C-type inactivation, and presumably the underlying constriction (Grissmer and Cahalan, 1989; Choi et al., 1991). The synthesis of results obtained from experiments that examined the role of position 449 residues on TEA potency and C-type inactivation led to a comprehensive and compelling model of TEA binding, and the mechanism underlying its influence on slow inactivation (Yellen, 1998). In this model, TEA was coordinated by the position 449 residues. During C-type inactivation, the position 449 residues moved closer together as a result of the constriction. However, the constriction could not occur if a TEA molecule was coordinated by these residues, and only resumed when TEA came off of its binding site. Thus, TEA slowed inactivation by a "foot-in-the-door" mechanism. Once the inactivation door was shut, external TEA could no longer bind to the channel.

4.6.4 Problems with the Model

Recent evidence, however, is incompatible with the accepted models of TEA binding and slow inactivation. Compared with results obtained in the *Shaker* channel, TEA potency in Kv2.1 is relatively little affected by mutations at the position equivalent to 449 (position 380 in Kv2.1) (Andalib et al., 2004). Introduction of cysteines into position 380 and 449 of Kv2.1 and *Shaker*, respectively, facilitated studies of the role of these residues in TEA binding. Covalent modification of these cysteines by the positively charged sulfhydryl reagent, MTSET, was unaffected by the presence of TEA in the outer vestibule (Andalib et al., 2004). These results clearly demonstrated that TEA binding does not involve direct coordination by the position 380/449 residues. Additional cysteine protection experiments suggested that TEA may occupy a location at a somewhat more external location in the outer vestibule (Andalib et al., 2004).

These cysteine protection experiments were also at odds with the proposed mechanism of slow inactivation. In fully inactivated *Shaker* channels, where the position 449 cysteines were presumed to have moved centrally within the conduction pathway, the presence of TEA in the outer vestibule did not prevent cysteine modification by MTSET (Andalib et al., 2004). However, TEA did inhibit modification of these cysteines by larger MTS reagents in fully inactivated channels, which demonstrated that TEA does bind to inactivated channels. Thus, these results argue against a model whereby the position 449 side chains move to a central location within the

conduction pathway during inactivation. Although these data do not rule out the possibility that TEA slows inactivation by a foot-in-the-door mechanism, they do indicate that the position 449 residues are not the door.

The results described above indicate that TEA does not bind where and how it had been thought to bind, and that the mechanism of C-type inactivation is not entirely understood. Indeed, there are many other apparently conflicting findings regarding slow inactivation. For example, whereas classical C-type inactivation, as observed in *Shaker* and the Kv1.3 potassium channel, is slowed by external TEA, slow inactivation in the Kv2.1 channel is virtually unaffected by external TEA. And whereas elevation of external K^+ slows classical C-type inactivation (López-Barneo et al., 1993), it accelerates slow inactivation in Kv2.1 (Immke et al., 1999). Interestingly, the silent α subunit, Kv9.3, has a dramatic influence on slow inactivation in Kv2.1, apparently by subtly changing channel structure near the cytoplasmic activation gate (Kerschensteiner et al., 2003). How this effect relates to slow inactivation at the selectivity filter is unclear. However, slow inactivation appears to proceed at different rates, and via different conformational routes, in closed and open channels (Klemic et al., 1998, 2001). The Kv9.3 effect near the cytoplasmic gate appears to shift the balance between these two routes (Kerschensteiner et al., 2003). These data suggest that, similar to activation, the slow inactivation mechanism at the selectivity filter may be energetically coupled to the cytoplasmic end of the pore. [An alternative possibility is that, despite its similarities, slow inactivation in Kv2 channels is mechanistically unrelated to C-type inactivation in Kv1 channels. Indeed, it has been postulated that Kv4 channels, which display a slow inactivation process that shares many similarities with Kv2, utilize a slow inactivation mechanism distinct from that of Kv1 channels (see Jerng et al., 2004a).] In summary, whereas it seems likely that slow inactivation involves some sort of constriction at or near the selectivity filter, the precise set of structural events that constitute inactivation, and whether inactivation results from just one or multiple mechanisms, remain unknown.

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4.6.5 Occupancy of the Outer Vestibule by Cations

In order for K^+ to enter the narrow selectivity filter, it must be almost completely dehydrated (similarly, K^+ exiting the selectivity filter must rehydrate). Crystallization of KcsA in the presence of potassium revealed an outer vestibule K^+ binding site that appears to be the location of K^+ dehydration/rehydration (Zhou et al., 2001b). In fact, it appears that this site may consist of two outer vestibule positions, a more external position where a more hydrated K^+ resides, and a position closer to the selectivity filter where a partially dehydrated K^+ resides. Biophysical data demonstrated that this site has important functional properties in at least one channel. In Kv2.1, a lysine (which is positively charged) located in the external portion of the outer vestibule interferes with the access of K^+ to this site (Consiglio et al., 2003). This interaction reduces single-channel conductance (Trapani and Korn, 2003), reduces K^+ permeability (Consiglio et al., 2003), and reduces the sensitivity of Kv2.1 currents to changes in external K^+ concentration (Andalib et al., 2002). Moreover,

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changes in outer vestibule conformation that accompany changes in external [K^+] reorient this lysine, which consequently changes the magnitude of its influence on channel conductance (Wood and Korn, 2000; Trapani et al., ~~2005~~). Interestingly, this binding site appears to be selective for K^+ and Rb^+; the interactions of Na^+ and Cs^+ with the channel are unaffected by either the presence or movement of the outer vestibule lysine (unpublished data).

The outer vestibule lysines also interfere with TEA binding, but do not interfere with access of the positively charged MTSET to cysteines deep within the outer vestibule. This suggests that the lysines don't simply electrostatically repel TEA but interfere specifically with the interaction of TEA with its binding site. However, TEA and external K^+ do not compete at concentrations that produce a measurable interaction of each with the outer vestibule (unpublished data). Whereas the simple explanation is that K^+ and TEA do not compete for the same cation binding site, this leaves one with the uncomfortable conclusion that there are two completely isolated cation binding sites in the outer vestibule. [Interestingly, external K^+ and TEA do not compete with each other in either Shaker or Kv2.1, but they do compete in Kv1.5 channels with the residue at position 480 (equivalent to Shaker 449) mutated to allow for high-affinity TEA binding.] Additional complexity is added with the observation that an intermediately sized cation, tetramethylammonium (TMA), apparently cannot occupy the outer vestibule in a functionally meaningful way (Andalib et al., 2004).

So, where and how are these cations binding? The results described above suggest that TEA binding does not involve direct coordination by position 380/449 residues in the outer vestibule. In addition, there are no other candidate residues to perform this role. Together, these observations suggest that TEA is more indirectly stabilized in the aqueous outer vestibule. This possibility is supported by molecular dynamics simulations, which suggest that TEA tumbles while at its "binding site" (Crouzy et al., 2001). However, this indirect stabilization mechanism must be selective for TEA; TMA is not similarly stabilized. Similarly, K^+ must be stabilized in the outer vestibule by a mechanism that apparently doesn't influence Cs^+ and Na^+. What is the nature of a cation stabilization site that is selective for one monovalent ion over another? Are TEA and K^+ stabilized differently? A better understanding of the outer vestibule would undoubtedly help toward the development of small molecules that reversibly enhance or block K^+ current through specific K^+ channels via actions in the external mouth of the pore.

4.7 Functions of the N-Terminal Domain

4.7.1 Fast Inactivation

The N-terminal domain (N-terminus) is most notably involved in the process of fast, or N-type, inactivation. The mechanism of N-type inactivation is well understood (Aldrich, 2001; Zhou et al., 2001a), and represents a puzzle that was solved by a series

of remarkably insightful investigations. Whereas many investigators contributed to filling in the pieces over the last 55 years (see Aldrich, 2001), four seminal studies provided the bulk of the information. Fast inactivation was initially postulated as an unknown mechanism by which the squid giant axon Na^+ conductance turned off during the action potential (Hodgkin and Katz, 1949). To explain their mathematical model of the Na^+ conductance underlying the squid axon action potential, Hodgkin and Huxley (1952) postulated the existence of "particles," three of which moved to initiate activation and one of which moved to produce inactivation. By combining a variety of physiological evidence with remarkable interpretation, Armstrong and Bezanilla (1977) proposed the "ball and chain" model of inactivation, whereby a charged inactivation ball (or particle) was tethered to the cytoplasmic end of the Na^+ channel by a polypeptide chain. Upon depolarization, the positively charged inactivation ball entered into the conduction pathway and blocked the pore (see Fig. 4.2). In the early 1990s, Aldrich and coworkers demonstrated the enormous power of applying molecular techniques to the study of cloned ion channels when they identified the inactivation ball and chain on the rapidly inactivating, Shaker potassium channel (Hoshi et al., 1990; Zagotta et al., 1990). In a series of elegant studies, these investigators demonstrated that, indeed, fast inactivation was mediated by a charged yet hydrophobic polypeptide "ball" located on the distal portion of the N-terminal domain of the channel. Finally, combined structural and biophysical studies illustrated the mechanism by which the inactivation "ball" enters and docks within the pore (Gulbis et al., 2000; Zhou et al., 2001a). Interestingly, fast, N-type inactivation can be conferred in one of two ways. Some channels, like Shaker, have the inactivation ball tethered directly to the N-terminus of the α subunit. Many K^+ channels, however, lack an intrinsic N-type inactivation mechanism and obtain one by associating with Kvβ subunits (Rettig et al., 1994; Zhou et al., 2001a).

4.7.2 Other Functions

In six transmembrane domain K^+ channels, the N-terminus also contains a molecular structure involved in channel assembly, called the T1 or tetramerization domain (cf. Shen et al., 1993; Long et al., 2005a). This domain appears to prevent the formation of heteromers among subunits derived from different K^+ channel families (Li et al., 1992; Shen and Pfaffinger, 1995). In addition, although it is not required for channel assembly (Kobertz and Miller, 1999), it does appear to facilitate channel formation (Zerangue et al., 2000).

Subtle mutations presumed to be near the interface between the T1 domain and the rest of the channel alter both activation voltage and deactivation rate (Cushman et al., 2000; Minor et al., 2000). The buried location of residues that influence gating suggests the possibility that, rather than being a modulatory domain, the T1 domain may be structurally close to the gating apparatus and mutations in the T1 domain alter gating for structural reasons. Some indirect evidence also supports the possibility that interactions between the N-terminus and the gating apparatus may be physiologically relevant. First, formation of heterotetramers between a silent α

subunit, Kv2.3, and functional Kv2.1 subunits, alter Kv2.1 channel gating apparently via a change in N-terminus properties (Chiara et al., 1999). Second, β subunits, which can alter channel gating properties, bind to the N-terminus (Gulbis et al., 2000; Long et al., 2005a). Nonetheless, other than its role in fast inactivation, the role of the N-terminus in K^+ channel physiology remains conjecture. For example, removal of the entire N-terminus has little effect on K^+ channel function (Kobertz and Miller, 1999). Moreover, mutagenesis and structural studies suggest that the N and C termini directly interact (Schulteis et al., 1996; Ju et al., 2003; Kuo et al., 2003; Sokolova et al., 2003), and it appears that binding of the β subunit to the N-terminus alters the interaction of C-terminal domains with the channel (Sokolova et al., 2003). Thus, experimental manipulations of the N-terminus may influence gating indirectly, via changes in the interaction of C-terminal domains with the channel.

4.8 Modulation at the C-Terminal Domain

Until recently, relatively little attention had been paid to the C-terminal domain (C-terminus) of the K^+ channel. Part of the reason for this probably relates to a lack of understanding of the structural relationship of the C-terminus to the rest of the channel. The recent determination of crystal structures for a Ca^{2+}-dependent K^+ channel (Jiang et al., 2002) and inward rectifier channels (Nishida and MacKinnon, 2002; Kuo et al., 2003) demonstrated, however, that the C-terminal domain was ordered, and formed a symmetrical tetrameric structure adjacent to the cytoplasmic end of the pore. An electron microscopic, low-resolution 3D structure of *Shaker* with and without a large portion of the C-terminal domain suggested that a compact C-terminal domain is juxtaposed to, and surrounding, the T1 domain of the N-terminus (Sokolova et al., 2003). This structural data has provided great insight into previous and subsequent functional data, and has opened the door to detailed mechanistic analysis of C-terminal function.

The length of the C-terminus varies greatly among K^+ channels, ranging from $<$100 amino acids to $\sim$450 amino acids in Kv channels. The C-terminus of the Ca^{2+}-dependent, BK channel, which is critical to its function, is $\sim$800 amino acids long. This variation by itself suggested that this channel domain must have some functional significance. Mutagenesis experiments on several K^+ channels have identified the C-terminus as the location of functionally relevant phosphorylation sites (Holmes et al., 1996; Murakoshi et al., 1997; Zilberberg et al., 2000; Sergeant et al., 2004). These studies suggested that, at the very least, the C-terminus is a conduit for biochemical modulation of K^+ channel behavior. However, many studies, including the few described below, suggest an even more central role of the C-terminus in K^+ channel function.

Specific point mutations in the C-terminus in *Shaker*, close to the S6 domain, influence both gating and permeation properties of the channel (Ding and Horn, 2002, 2003). These dual influences suggested that this region is near the cytoplasmic entrance to the pore, and can affect the operation of the cytoplasmic gate.

Mutation-induced shifts in voltage dependence further suggested that this region is relevant to the coupling between the voltage sensor and the cytoplasmic gate (Ding and Horn, 2003). This interpretation was supported by subsequent work on the BK family of K^+ channels (see below; Ca^{2+}-dependent K^+ channel structure and function are described in detail in Chapter 6).

Several elegant studies of the mechanism of Ca^{2+} dependence of the BK channel have provided great insight into the role of the C-terminus in regulation of channel function. BK channels respond to Ca^{2+} at concentrations that range over five orders of magnitude (Xia et al., 2002). To achieve this range of sensitivity, BK channels have at least two different Ca^{2+} regulatory sites in the C-terminus (Xia et al., 2002) and possibly one located elsewhere (Piskorowski and Aldrich, 2002). Remarkably, the C-terminus appears to be a completely modular channel domain. The BK channel homolog, mSlo3, responds not to intracellular Ca^{2+} but rather, to changes in intracellular pH (Schreiber et al., 1998). Swapping the C-terminal domains between BK and mSlo3 completely exchanged ligand sensitivity (Xia et al., 2004). Thus, the C-terminus of mSlo3 conferred pH sensitivity (and lack of Ca^{2+} sensitivity) on BK and vice versa. These results suggest that the C-terminal domains from different channels, which respond to different modulatory ligands, are completely exchangeable. Interestingly, both ~~mSlo~~ channels contain an identical sequence that links the C-terminus to the S6 domain. This linker region corresponds to the region in voltage-gated K^+ channels, studied by Ding and Horn (2003), that influences the coupling between the voltage sensor and the cytoplasmic gate. Taken together, these results suggested that changes in C-terminus conformation may influence gating via a direct action on the cytoplasmic gate. Based on structural data, Jiang et al. (2002) suggested a mechanism for this effect, whereby a Ca^{2+}-induced change in conformation of the C-terminus was mechanically coupled to opening of the cytoplasmic gate.

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Tests of this hypothesis continued with a set of experiments by Niu et al. (2004), who demonstrated that changes in the length of this linker altered the response of BK channels to Ca^{2+}. In the presence of Ca^{2+}, shortening the linker increased, and lengthening the linker decreased, the probability of channel opening in response to voltage. Niu et al. (2004) proposed a model whereby the C-terminus was connected to the cytoplasmic gate by a spring mechanism, and that changing linker (spring) length, and therefore tension, changed the probability of channel opening at all voltages.

In summary, current models suggest that the C-terminus is a modular channel domain, which exerts mechanical force on the S6 domain in the region of the cytoplasmic gate. In some channels, alteration of this force is directly responsible for opening the gate, whereas in others, it changes the probability of opening in response to another, primary stimulus (e.g., voltage). This model also appears to hold for channels that use the selectivity filter as a gate (described above). For example, in CNG channels, interaction of cyclic nucleotides with the C-terminus produces a conformational change in the region of the cytoplasmic gate, which is associated with opening of the selectivity filter gate (Flynn and Zagotta, 2001; Johnson and Zagotta, 2001). Conversely, interaction of ATP with the C-terminus of the KATP

channel closes the selectivity filter gate (Proks et al., 2003). Thus, the great variety of C-terminal structures may largely reflect the need for channels to respond to different ligands and/or modulators. Within this context, the C-terminus may play a generally common role, and act by a fundamentally common mechanism, in many channels.

4.9 The MinK/MiRP Family of Accessory Subunits

The functional properties of voltage-gated K^+ channels are altered by a wide variety of accessory proteins, broadly called β subunits. Co-assembly with β subunits influences trafficking, cell surface expression, and physiological properties of α subunits. The Kvβ class of subunits are cytoplasmic proteins that bind to the N-terminal domains in a fourfold symmetric configuration (Zhou et al., 2001a), and will be discussed in Part III. Another class of β subunits, the MinK/MiRP peptides, are integral membrane proteins with one membrane spanning region (Fig. 4.3A). In a variety of cells, complexes composed of K^+ channel α subunits and MinK/MiRP peptides create a specific endogenous K^+ current. For example, the cardiac I_{Ks} ~~channel~~ is derived from the KvLQT channel (the α subunit) and MinK peptide (Fig. 4.3A–C; Barhanin et al., 1996; Sanguinetti et al., 1996). Similarly, a skeletal muscle K^+ channel is derived from co-assembled Kv3.4 subunits and MiRP2 peptides (Fig. 4.3D–G; Abbott et al., 2001). Moreover, a variety of serious, often fatal diseases are associated with point mutations in one or another member of the MinK/MiRP peptide family (cf. Goldstein et al., 2004). For example, several variants of cardiac long Q-T syndrome, a cardiac arrhythmia that often leads to a fatal ventricular fibrillation, are associated with mutations to MinK/MiRP peptides (Abbott et al., 1999, 2001; Abbott and Goldstein, 2002; Goldstein et al., 2004). Mutations in one family member, MiRP1, are responsible for a common form of drug-induced arrhythmia, which often leads to sudden death (Abbott et al., 1999, Sesti et al., 2000).

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Whereas their importance has led to extensive investigation, the mechanisms by which they modify α subunits remain obscure. The complexity of this interaction can be illustrated by just a few examples. Co-assembly of the MiRP2 peptide with the Kv2.1 and Kv3.1 potassium channels reduces current density and slows activation rate, but produces *no change* in voltage dependence of current activation (McCrossan et al., 2003). In contrast, co-assembly of MiRP2 with the Kv3.4 potassium channel shifts the voltage dependence of current activation in the *negative direction* by almost 40 mV (Abbott et al., 2001). Yet another contrasting effect is observed with the co-assembly of MiRP1 with the Kv4.2 channel. As with most other MiRP—α subunit interactions, co-assembly of MiRP1 with Kv4.2 slows activation (Zhang et al., 2001). However, MiRP1 also shifts the voltage dependence of Kv4.2 activation in the *positive direction* by about 30 mV (Zhang et al., 2001). Finally, many MinK/MiRP interactions with α subunits result in a substantial change in inactivation rate. As discussed above, activation and inactivation mechanisms are

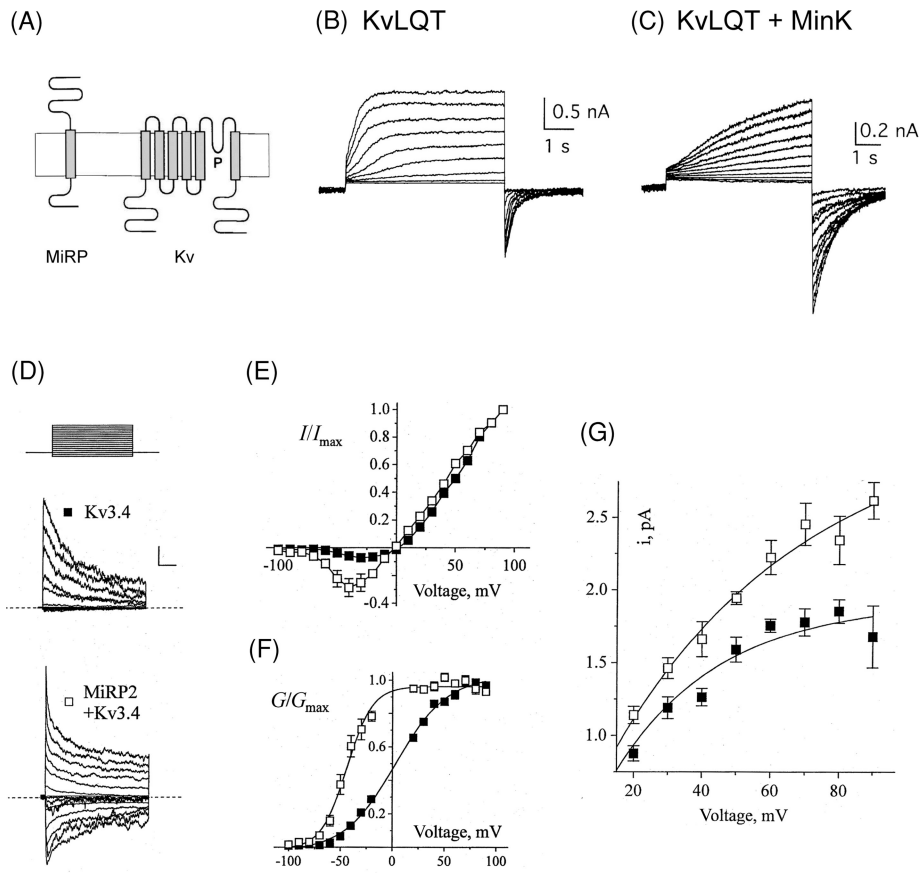

Fig. 4.3 Changes in a channel properties by co-assembly with a MinK/MiRP accessory subunit. (A) Schematic representation of a single transmembrane domain MinK/MiRP subunit and a six transmembrane domain α subunit. (B–C) K^+ currents through channels composed solely of KvLQT1 channel subunits (B) or channels composed of co-assembled KvLQT1 + human MinK subunits (C). Note the dramatic (18-fold) slowing of activation in the co-assembled channel. Co-assembly with MinK also increases single-channel conductance ∼4-fold, shifts the voltage dependence of activation ∼25 mV in the positive direction and slows channel closing rate by ∼3-fold (Sesti and Goldstein, 1998). (D–G) Currents through channels composed solely of Kv3.4 and through channels composed of co-assembled Kv3.4 plus MiRP2. Currents were recorded with 100 mM internal and external K^+. Co-assembly with MiRP2 produces a dramatic change in slope of the activation curve (panel F), which results in the generation of large currents at very negative potentials (panels D, E). The inward current in the co-assembled channels results from the negative shift in the activation curve. Co-assembly with MiRP2 also produces a significant increase in single-channel conductance (panel G). These two effects would not only enhance the subthreshold current, but would tend to dramatically dampen excitation by producing a substantial K^+ conductance at the resting membrane potential. Currents in panels B–C were recorded in oocytes, currents in panel D were recorded in CHO cells. Figure kindly provided by Steve A. N. Goldstein. Adapted from Sesti and Goldstein, 1998 (panels B–C) and Abbott et al., 2001 (panels A, D–G).

structurally distinct. In sum, these small peptides have multiple effects, and the same peptide can have different effects on different channels.

Some headway has been made into understanding the structural interaction between the MinK/MiRP peptides and K^+ channel α subunits. It appears that two MinK subunits co-assemble with the tetrameric KvLQT1 channel (Chen et al., 2003a,~~b~~), although others have come to different conclusions (Tzounopoulos et al., 1995; Wang et al., 1998). It also appears that MinK is exposed to both the internal and external conduction pathway (Wang et al., 1996; Sesti and Goldstein, 1998; Tai and Goldstein, 1998; Chen et al., 2003~~a~~,b). These observations raise two questions. First, how do two MinK peptides fit within the conduction pathway of a symmetric channel composed of four α subunits, without disrupting the precisely tuned selectivity mechanism? Second, how does a small peptide that is exposed to the conduction pathway produce dramatic and widely differing effects on both activation and inactivation in different channels? Indeed, the complex and contrasting effects of these small peptides on α subunit function is currently perplexing. However, once the structural interaction between the different subunits is understood, the complex array of functional effects of these small peptides will undoubtedly provide new insight into the gating mechanisms, and modulation of gating mechanisms, of K^+ channels.

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Part III. Specific Properties of Voltage-Gated Channels

4.10 Diversity of Function

The complexity of the brain is derived from the integration of an enormous number of intracellular and intercellular events, occurring both simultaneously and sequentially on timescales of milliseconds to seconds. Individual neurons can have one or more specific functions within a circuit, depending on environmental and/or circumstantial events. To accomplish their different functions, a large number of ion channel species is required within a cell, and different combinations of ion channel species are required across different cell types. Some neurons are merely conduits for faithful transmission of incoming information, and the type of information being transmitted will dictate the need for specific channel types. Other neurons must integrate a variety of inputs, inhibitory and excitatory, of large and small magnitude, occurring at different locations on the cell at different times. This integration results in a constant decision-making process by the neuron as to whether and how to respond to the everchanging set of inputs. This within-neuron integration not only requires a large cohort of channels, but also requires that they can be modulated by a diverse array of second messengers and protein partners. The interaction of channels to form a functional result is extraordinarily complex. Indeed, it would be foolishly simplistic to assign a particular role to a particular channel in shaping neuronal excitation. Nonetheless, phenotypic differences among channels can generally be associated with one or more specific functional roles.

Stephen J. Korn and Josef G. Trapani

The heart represents a system in which the precise contribution of a multitude of channels to function is well understood (one can find many good, comprehensive descriptions in textbooks and reviews). In contrast, we are only beginning to understand how different K^+ channels coordinate and regulate neuronal behavior. Historically, identification of individual channel roles was examined with pharmacological manipulations. Although this approach, which continues today, has generated significant insight into how different channels shape intra- and intercellular function, an obvious shortcoming is the lack of specificity of available pharmacological agents. Several recent advances, most of which revolve around molecular technology, seem to be the final requirement for a comprehensive description and precise understanding of the role of channel species in neuronal electrophysiology. For example, the ability to generate knockout mice allows the study of circuitry in a system devoid of a single-channel species or modulator. Conversely, knock-in technology allows the expression of exogenously-provided channel species into a neuron. Even more promising is the burgeoning ability to direct expression (or lack of expression) to particular parts of a circuit, in vivo, using cell-specific promoters. This type of approach will lead to a comprehensive understanding of the role of the particular channel in cellular behavior. Moreover, it will ultimately reveal whether the influence of a channel mutation in a particular circuit (or cell) was necessary and sufficient to produce an entire disease, or one or more symptoms of a disease. The application of this technology to circuitry, especially in vivo, is just beginning to occur. In the following section, several examples are provided to illustrate how channel phenotype influences the electrophysiological behavior of a neuron at a cellular level. It must be stressed that these studies are also in an early stage, and even in these examples, our understanding of cellular behavior is incomplete.

4.11 Kv1 Channels

Kv1 channels are widely distributed, and have different functions in different neuronal circuits. Mutations to the Kv1.1 channel are most notably associated with episodic ataxia, which in turn, is associated with many other symptoms (Browne et al., 1994; Klein et al., 2004). To date, how a single mutation to a single channel leads to a diverse set of disease symptoms, or whether, in fact, all of the symptoms are associated with a single Kv1.1 mutation, are unknown.

Kv1.1, Kv1.2, Kv1.5, and Kv1.6, and/or heteromers derived from these channel subunits, can all be present in an individual neuron, and can all regulate subthreshold excitability (a measure of the likelihood that a cell will fire an action potential in response to a small, subthreshold stimulus). Indeed, when studied in isolation, multiple channels may make indistinguishable contributions to membrane currents. Upon closer scrutiny, however, one channel type may contribute to very different aspects of membrane excitability. Specialized functions can be based on cell type, membrane localization, stimulus characteristic, environment or physiological circumstance. Just a few examples will be given to provide a flavor for the association

of phenotype with function. Below are two examples of Kv1.1 function, not associated with ataxia, and an example of Kv1.3 function in neuronal electrophysiology.

Auditory neurons of the medial nucleus of the trapezoid body (MNTB) are involved in localizing sound from binaural intensity differences. To accomplish this task, neurons must respond with high-fidelity to high-frequency stimulation. For example, 20 stimuli generated at a very high frequency (hundreds of Hz) should result in 20 action potentials fired with exquisite temporal accuracy relative to the stimuli. MNTB neurons contain Kv1.1, Kv1.2, and Kv1.6 channels, most likely in heteromeric configurations (Brew et al., 2003). Both homomers and heteromers containing Kv1.1 activate at very negative membrane potentials (and thus generate a subthreshold current). Subthreshold currents have several functions. In MNTB neurons, they rapidly terminate a depolarization associated with a synaptic event, and thus limit the number of action potentials (hopefully to one) produced by the individual synaptic stimulus (Brew et al., 2003). In other neurons, subthreshold K^+ currents effectively dampen excitability by opposing the firing of action potentials by small stimuli (cf. McKay et al., 2005 and references within). Knock-out of Kv1.1 reduces subthreshold K^+ current activated at very negative voltages, perhaps by two mechanisms: by reduction of current and also by a positive shift in activation voltage for the current carried by other channel subunits that no longer form with Kv1.1 (cf. Brew et al., 2003). The reduction or loss of subthreshold current, in turn, leads to the firing of multiple action potentials in response to individual stimuli. Thus, binaural encoding of auditory information, which requires high-fidelity pairing of action potentials to stimuli, is made possible by the Kv1.1 channel. However, this Kv1.1 function is not by itself sufficient to ensure temporal fidelity at high frequencies. High-fidelity firing in auditory neurons also requires Kv3 channel function, which will be discussed below.

A second example involves Kv1.1 with a completely different personality. In MNTB cells, Kv1.1 produces a noninactivating outward current. However, Kv1.1 channels can couple to a β subunit, Kvβ1.1, to produce a rapidly inactivating current (Rettig et al., 1994; several Kv1 channels interact with one of three Kvβ subunits). This fast inactivation results from a standard ball and chain mechanism associated with N-type inactivation. In this case, however, the Kvβ subunit provides the inactivation ball to an α subunit that doesn't have one (cf. Zhou et al., 2001a). When coupled, Kv1.1/Kvβ1.1 channels activate at quite negative voltages (the Kv1.1 supplied property) and inactivate rapidly (the Kvβ1.1 property). Rapidly inactivating K^+ currents have a variety of roles, some of which will be discussed further below. But why create a new rapidly inactivating channel when several Kv1 and Kv4 channels have an intrinsic fast inactivation mechanism?

One electrophysiological event that involves rapidly inactivating K^+ channels composed of Kv1.1/Kvβ1.1 is spike broadening. If a rapidly inactivating K^+ channel is involved in repolarization of the action potential, then with high-frequency stimulation, successive action potentials will become longer in duration as K^+ channels inactivate (cf. Giese et al., 1998). This would occur regardless of the channel species utilized to produce the rapidly activating, rapidly inactivating current. However, the

precise nature of the electrophysiological event will differ in important ways depending on the channel species involved. For example, the fast inactivation process resulting from Kv1.1/Kvβ1.1 coupling will respond differently to high-frequency firing than will a channel with an intrinsic N-type inactivation mechanism. As action potentials broaden, Ca^{2+} influx during the action potential will increase. As a result, Ca^{2+}-dependent K^+ currents, which mediate slow afterhyperpolarizations, will be increased in magnitude and perhaps prolonged. This will reduce the likelihood of firing and/or reduce firing frequency. In addition, increasing Ca^{2+} influx will produce larger Ca^{2+}-dependent biochemical responses, and also, perhaps, increase the spread of intracellular Ca^{2+}. This will delocalize Ca^{2+}-dependent events. If K^+ channel inactivation increases further, perhaps via increased firing frequency, the magnitude of Ca^{2+} influx would continue to increase. However, when the rapidly inactivating K^+ current is derived from the Kv1.1/Kvβ1.1 complex, elevation of intracellular Ca^{2+} will inhibit the fast inactivation via a specific action on the N-terminal domain of Kvβ1.1 (Jow et al., 2004; see Fig. 4.9 for a similar effect involving a Kv4.2/frequenin complex). This reduction in inactivation would then lead to shorter action potentials, a reduction of Ca^{2+} influx and consequently a reduction in the magnitude or spread of Ca^{2+}-dependent processes. Thus, because Kvβ1.1 is Ca^{2+} sensitive, utilizing the Kv1.1/Kvβ1.1 complex to produce fast inactivation creates a feedback loop that can strike a balance between action potential broadening and shortening, and consequently place a limit on Ca^{2+} influx. In contrast, the inactivation rate of the Kv1.4 channel (which has an intrinsic ball and chain) is insensitive to Ca^{2+}. Consequently, if Kv1.4 were utilized as the rapidly inactivating channel, action potentials might broaden, but this process would be insensitive to Ca^{2+} influx and therefore not subject to feedback inhibition or modulation (Jow et al., 2004).

Another example of specialized function in the Kv1 family involves the Kv1.3 channel. The Kv1.3 channel is best known for its role in T-lymphocyte function, where it is involved in cytokine release. Indeed, this function of Kv1.3 is of clear clinical importance, and makes Kv1.3 a potentially important target for immunosuppression therapy (Chandy et al., 2004; Damjanovich et al., 2004; Valverde et al., 2005). But whereas Kv1.3 function is fairly straightforward in a lymphocyte, it can be a nexus for great complexity in other systems. For example, the Kv1.3 channel contributes significantly to the depolarization-activated K^+ current in olfactory neurons (Fadool and Levitan, 1998). Certainly, the simple activation of a large Kv1.3 current contributes significantly to the cell's firing behavior. However, this channel also appears to play a far more interesting and complex role in olfaction. Among the several Kv channels present in olfactory neurons, Kv1.3 is the site of modulation by multiple intracellular signals, many of which act via tyrosine kinases (Bowlby et al., 1997; Fadool and Levitan, 1998). Thus, in addition to altering action potential characteristics, deletion of Kv1.3 channels from olfactory neurons eliminates the ability of olfactory neurons to be modulated by biochemical signals that act via tyrosine kinase (Fadool et al., 2004). Moreover, elimination of Kv1.3 also results in an increased expression of several protein partners. Thus, not only have multiple cellular modulation mechanisms that converge on one particular ion channel

been eliminated, but it is likely that an untold number of previously unrelated events are also indirectly influenced. For example, the elevation of expression of interacting proteins, concomitant with elimination of the Kv1.3 target, would likely lead to a greater interaction of these proteins with another target. Finally, knock-out of Kv1.3 resulted in significant structural alterations in the olfactory bulb (Fadool et al., 2004). In summary, Kv1.3 knockout changed action potential shape, duration and firing frequency, changed the structure of the olfactory bulb, eliminated the ability to modulate the olfactory neurons by a large number of modulators and changed expression of a large number of proteins that interact with both Kv1.3 and other effector proteins. Clearly, the Kv1.3 channel plays many roles in olfactory neurons.

A surprising consequence of these changes is that mice lacking Kv1.3 channels were better at detecting and discriminating odors. The opportunities for understanding channel function, and neuronal function, raised by this finding are many. One would assume that better odor detection and discrimination are good things for a mouse, yet elimination of a dominant channel in olfactory neurons apparently made olfactory function better. One might argue, from an evolutionary point of view, that the presence of Kv1.3 in the olfactory bulb, where it apparently reduces odor detection capabilities, provides an adaptive advantage. It remains to be determined what advantage has been gained, how this single ion channel influences the tradeoff of advantages and disadvantages, and indeed, how olfaction works!

4.12 Kv2 Channels

There are just two functional members of the Kv2 family, Kv2.1 and Kv2.2. To the extent that they have been compared, these channels are quite similar in both sequence and physiological characteristics. Kv2 channels have relatively long carboxy-termini for Kv channels, which suggests one or more roles for this region in channel function. These channels display differences in expression during development, apparently due to differences in the carboxy-terminal sequence of the channel (Blaine et al., 2004). Kv2.1 is present in many neuron types throughout the nervous system, as well as many peripheral organs, such as heart and pancreas. Kv2.2 channels are predominantly found in smooth muscle, but can also be found in a variety of neurons. Finally, despite the similarity of function, these two channels seem to have somewhat different roles in influencing cell excitability (cf. Malin and Nerbonne, 2002; Blaine et al., 2004).

Kv2.1 channels activate more slowly than Kv1 channels, and display a slow inactivation (Fig. 4.4). As with all other Kv channels, Kv2.1 serves a variety of purposes, depending on its relative abundance, its cellular location and the complement of other channels in the particular neuron. One unique role of Kv2.1 has been demonstrated in hippocampal neurons. The contribution of Kv2.1 channels to the overall current in hippocampal neurons is quite small (Mitterdorfer and Bean, 2002). Nonetheless, ∼90% knock-down of Kv2.1 has a dramatic and somewhat surprising effect (Du et al., 2000). Consistent with its limited contribution to the total K^+

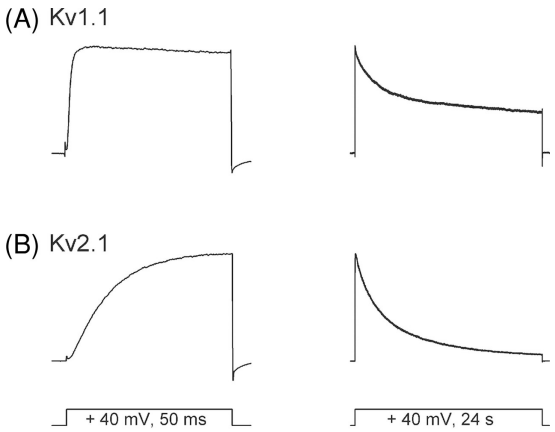

Fig. 4.4 Differences in activation and inactivation rates in Kv1.1 and Kv2.1. Kv1.1 activates rapidly and, with prolonged activation, inactivates relatively little (panel A). In contrast, Kv2.1 activates much more slowly and completely inactivates with prolonged activation (panel B). Recordings from HEK cells by Josef G. Trapani and Payam Andalib.

current, the duration of single action potentials is virtually unaffected by the absence of Kv2.1. However, at slightly elevated extracellular [K^+] (8.5 mM), which causes hippocampal neurons to fire action potentials in bursts, action potential duration is prolonged more than 10-fold in the absence of Kv2.1 channels (Fig. 4.5). Thus, it appears that the primary role of Kv2.1 in hippocampal neurons is to maintain action potential integrity when extracellular [K^+] is elevated.

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Kv2.1 has an interesting and unique property that may underlie this physiological role. Upon elevation of external [K^+], both current magnitude and activation rate are increased (Fig. 4.6A; Wood and Korn, 2000; Consiglio and Korn, 2004). The effect on current magnitude is opposite to that expected given the reduction in electrochemical driving force, and opposite to that seen for most all other K^+ channels (Fig. 4.6B; the HERG K^+ channel, which is most notable for its role in heart physiology, also displays an anomalous increase in current upon [K^+] elevation, which is produced by a different mechanism). Thus, under high-frequency firing situations, when extracellular [K^+] might rise, current through Kv2.1 apparently increases while current through other channels decreases. This would serve to maintain total K^+ current density during an action potential, and thus maintain action potential integrity.

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The molecular mechanism that underlies this effect was described in a previous section of this chapter. Briefly, Kv2.1 can open into one of two conformations. At lower [K^+], some channels open into a high-conductance state and some open into a lower conductance state. The fraction of channels in each conformation depends on the occupancy of a specific K^+ binding site in the selectivity filter when the channel opens, which in turn is dependent on the external [K^+] (Immke et al., 1999; Immke and Korn, 2000). At higher [K^+], a larger fraction of channels open

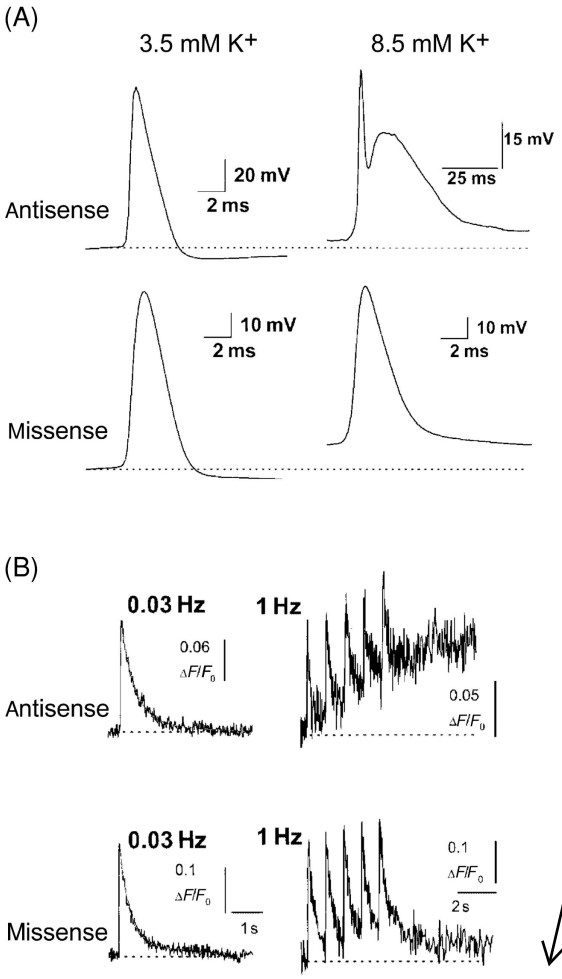

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Fig. 4.5 K^+-dependent potentiation of current through the Kv2.1 channel. Elevation of external $[K^+]$ would be expected to reduce outward current magnitude due to the reduction in electrochemical driving force. Such an effect, which happens in virtually all K^+ channels, is illustrated for Shaker in panel B. Interestingly, the magnitude of the reduction for a given change in driving force differs in different channels. In Kv2.1, however, the net effect of increasing external $[K^+]$ between 0 and 10 mM is an increase in outward current (panel A). This reflects two special characteristics of Kv2.1. First, current through the channel responds very little to the change in driving force (Andalib et al., 2002). Second, the outer vestibule of the channel can open into one of two conformations, one of which has ~4-fold higher conductance than the other (Immke and Korn, 2000; Trapani et al., ~~2005~~). At higher $[K^+]$, more channels open into the higher conductance conformation (Wood and Korn, 2000). Note also that elevation of external $[K^+]$ speeds inactivation in Kv2.1 and slows inactivation in Shaker. Currents were recorded in HEK cells.

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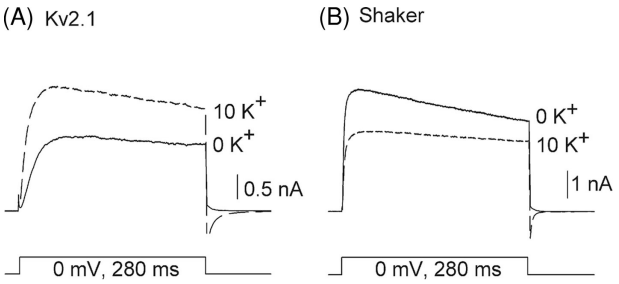

Fig. 4.6 Role of Kv2.1 in high-frequency firing of action potentials. (A) Action potentials were recorded from pyramidal cells in hippocampal slices at two external [K^+]. Slices were exposed either to an antisense oligonucleotide to knock down Kv2.1 expression by ∼90% or a missense oligonucleotide, which did nothing to Kv2.1 expression. At normal physiological [K^+] (3.5 mM), knockdown of Kv2.1 expression had little or no effect on action potential duration. However, at high external [K^+] (8.5 mM), which leads to bursts of action potentials, action potential duration was greatly prolonged (note the time scale difference). (B) Intracellular Ca^{2+} transients, evoked by 0.3 Hz or 1 Hz stimulation. With Kv2.1 present, Ca^{2+} transients rise and fall with each stimulus, regardless of frequency. At higher frequency, however, the absence of Kv2.1 leads to a steady rise in [Ca^{2+}] that is uncoupled from the stimulus. Figure kindly provided by Chris McBain, and adapted from Du et al. (2000).

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into the higher conductance state, and consequently, total macroscopic K^+ current through Kv2.1 channels is larger. Recent evidence indicates that the single-channel conductance of the lower conductance state is ∼1/4 that of the higher conductance state (Trapani et al., ~~2005~~). In heterologous expression systems, only 10–20% of channels are in the low-conductance state at physiological [K^+], but conversion of these channels to the high-conductance state can have a significant impact on current magnitude. This raises an interesting issue for the in situ situation. Hippocampal neurons display a significant cell surface expression level of Kv2.1 (Misonou and Trimmer, 2004), yet, as mentioned above, Kv2.1 generates little current when single action potentials are evoked (Mitterdorfer and Bean, 2002). This may reflect two Kv2.1 properties. First, they activate and deactivate relatively slowly. Consequently, if action potentials fire more rapidly than channels deactivate, Kv2.1 current will grow with each succeeding action potential. Second, the observed function of Kv2.1 raises the intriguing possibility that, at normal physiological [K^+], a large number of Kv2.1 channels are in the low-conductance state. If so, the conformationally-based difference in conductance must be regulated by factors other than [K^+].

Kv2.1 channels have an additional pair of properties that appear to be linked: they cluster on the membrane surface and they are constitutively phosphorylated under resting conditions. Dephosphorylation results in a negative shift in the voltage dependence of activation and a dispersion of Kv2.1 (Misonou et al., 2004). Both dephosphorylation and dispersion are caused by glutamate exposure, which suggests that excitation is the responsible stimulus (Misonou et al., 2004). The negative shift in activation would tend to reduce membrane excitability. The role of clustering and dispersion are unknown. It is interesting, however, that the Kv2.1 clusters tend to be

located on membranes apposed to astrocytic processes (Du et al., 1998). Astrocytes release glutamate in response to neuronal activity (cf. Araque et al., 2000), and appear to be actively involved in shaping neuronal events (cf. Fellin et al., 2004). Moreover, astrocytes have long been known to function as external [K^+] buffering systems. This raises the intriguing possibility that a dynamic set of events occur that involve high-frequency firing, external [K^+] buffering, and current magnitude through [K^+]-sensitive Kv2.1 channels.

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4.13 Kv3 Channels

There are four Kv3 channels, Kv3.1–Kv3.4. Two of these channels display fast inactivation, two do not (Fig. 4.7). Kv3.1 and Kv3.2 channels have perhaps the most well-defined role in neuronal physiology (the primary difference between these two channels is their ability to be modulated ~~by protein kinase A~~). They are designed to allow action potentials to fire rapidly and with high fidelity. As described earlier, this accurate high-frequency behavior requires Kv1 channels, which produce subthreshold currents, limit action potential firing in MNTB neurons to one per stimulus and lock action potential generation to the synaptic stimulus (Kaczmarek et al., 2005). However, another K^+ channel function is also required to ensure fidelity of rapidly firing action potentials: the channel responsible for repolarization of the action potential must (1) activate rapidly, (2) activate only at relatively positive membrane potentials, (3) carry a large current, and (4) close very quickly upon hyperpolarization. This function is supplied by Kv3 channels. Because Kv3 channels only activate at positive membrane potentials, they won't activate until well above action potential threshold, and consequently, won't interfere with action potential generation. The lack of subthreshold activation, combined with fast activation of a large current by strong depolarizations, permits the rapid activation, followed by rapid termination of the action potential. These biophysical properties keep action potentials very brief and associated temporally with the stimulus (see Fig. 4.7). Fast deactivation (channel closing) is also required because the succeeding action potential in a train would be delayed or inhibited by any residual K^+ conductance. Clearly, one would not want a channel such as Kv2.1 to be present in neurons whose function is to rapidly fire a large number of action potentials with a highly accurate temporal association with a stimulus. The slow activation of Kv2.1 would result in a broader action potential and the slow closing of Kv2.1 would prevent or delay the firing of the subsequent action potentials.

Kv3 channels are also substrates for a large number of modulators (see Rudy and McBain, 2001). As an example, Kv3.1 is phosphorylated by protein kinase C in MNTB neurons under resting conditions. Dephosphorylation can result in up to a −40 mV shift in the voltage dependence of activation, which would result in the activation of a larger current with depolarization. Modulation of Kv3.1 current magnitude by channel phosphorylation has a significant impact on both firing rate (smaller Kv3.1 currents decrease firing rate, as expected) and the fidelity with which action potentials follow high-frequency stimuli (Kaczmarek et al., 2005). The elegance of

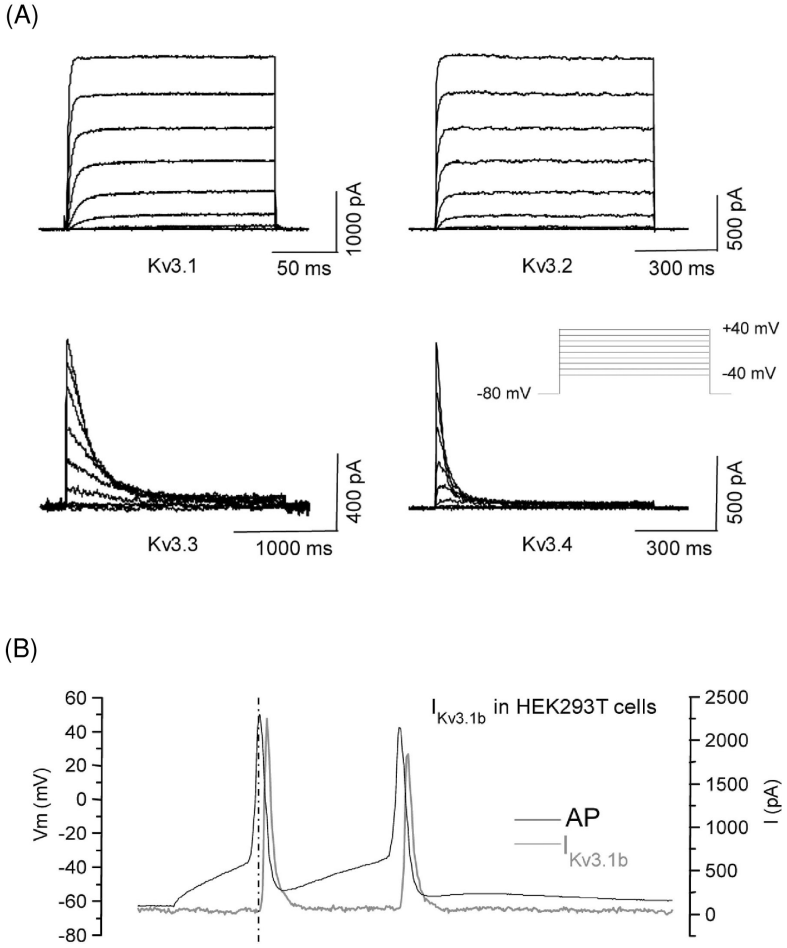

Fig. 4.7 Properties of Kv3 channels. There are four members of the Kv3 channel family, all of which open at relatively positive membrane potentials ($V_{1/2} = \sim +10$ mV). All channels activate and close rapidly. Kv3.1 and Kv3.2 don't inactivate, Kv3.3 inactivates relatively quickly and Kv3.4 inactivates even more quickly (panel A). In some neurons, Kv3.1 and Kv3.4 subunits co-assemble, resulting in a potassium channel that produces a faster hyperpolarization, decreased action potential duration and enhanced ability to fire with high frequency (Baranauskas et al., 2003). Indeed, formation of heteromeric channels with different subunit stoichiometry may provide a means to modify Kv3 channel function to optimally support action potential firing over a given frequency range (Li et al., 2001; Baranauskas et al., 2003). Panel B illustrates the activation of Kv3.1b currents by waveforms created to mimic the firing of a neocortical neuron. Note that the current activates late during the action potential (due to the positive $V_{1/2}$) and closes extremely rapidly. Figure kindly provided by Chris McBain, and adapted from Rudy and McBain (2001).

this modulation can be observed in the fine tuning of MNTB neuron physiology: high-frequency auditory stimulation causes dephosphorylation of Kv3.1 in MNTB neurons, which consequently increases Kv3.1 current magnitude and enhances the ability of the neuron to fire at high frequencies (Song et al., 2005).

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4. Voltage-Gated Potassium Channels

The well-described roles of Kv1 and Kv3 channels in MNTB neurons provide a good example of how each kinetic component of channel behavior, together with selective modulation of a particular channel's properties, can produce an optimum result for a needed neuronal function. Additional examples of the fine-tuning of fast spiking behavior by Kv3 channels are described in an excellent older review (Rudy and McBain, ~~2000~~) and more recent papers (cf. Baranauskas et al., 2003; Lien and Jonas, 2003; Goldberg et al., 2005).

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4.14 Kv4 Channels

There are three members of the Kv4 family, Kv4.1–Kv4.3, which have similar biophysical properties and pharmacology (Jerng et al., 2004a). These channels, whose hallmark is their rapid inactivation (Fig. 4.8A), are now believed to underlie the classical, transient K^+ current, I_A, first described in gastropod neurons over 30 years ago (Connor and Stevens, 1971a~~,b~~). Whereas they were originally shown to play a role in the timing of rhythmic firing behavior (Connor and Stevens, 1971a~~,~~b), they have since been shown to play an important role in a variety of electrophysiological events, including control of action potential duration, timing of responses to multiple synaptic inputs, and long-term potentiation (Schoppa and Westbrook, 1999; Mitterdorfer and Bean, 2002; Watanabe et al., 2002; Jerng et al., 2004a).

4.14.1 Influence of Kv4.2 on Hippocampal Dendrite Physiology

One function that is uniquely provided by the properties of Kv4 channels is that of regulating dendritic excitability in hippocampal pyramidal neurons. Kv4.2 is localized in dendritic membranes, with an increasing gradient of current density from proximal to distal segments (Hoffman et al., 1997). An apparently important contributor to hippocampal neuron function is the backpropagation of action potentials from the soma into the dendritic tree. The increasing gradient of Kv4.2 channels from proximal to distal dendrites causes the amplitude and number of back propagating action potentials to diminish with distance from the soma. The presence of Kv4.2 channels in distal dendrites also reduces the amplitude of excitatory postsynaptic potentials (epsps) originating from distal afferents (Hoffman et al., 1997). Most importantly, because of the rapid inactivation of Kv4.2 channels, an initial depolarization event can alter the membrane response to a second event that immediately follows. Thus, with appropriate timing, subthreshold epsps can reduce I_A and consequently increase the amplitude of closely following epsps or action potentials that are backpropagating into the distal dendrites (Johnston et al., 2000). Both epsp potentiation and the latter process, called action potential "boosting," may have a profound role in learning and memory. Long-term potentiation (LTP), which has long been studied as an electrophysiological mechanism of neuronal plasticity, is a process wherein synaptic responses to moderate stimuli are enhanced for a prolonged period by a single, preceding, powerful stimulus. Inhibition of I_A in hippocampal

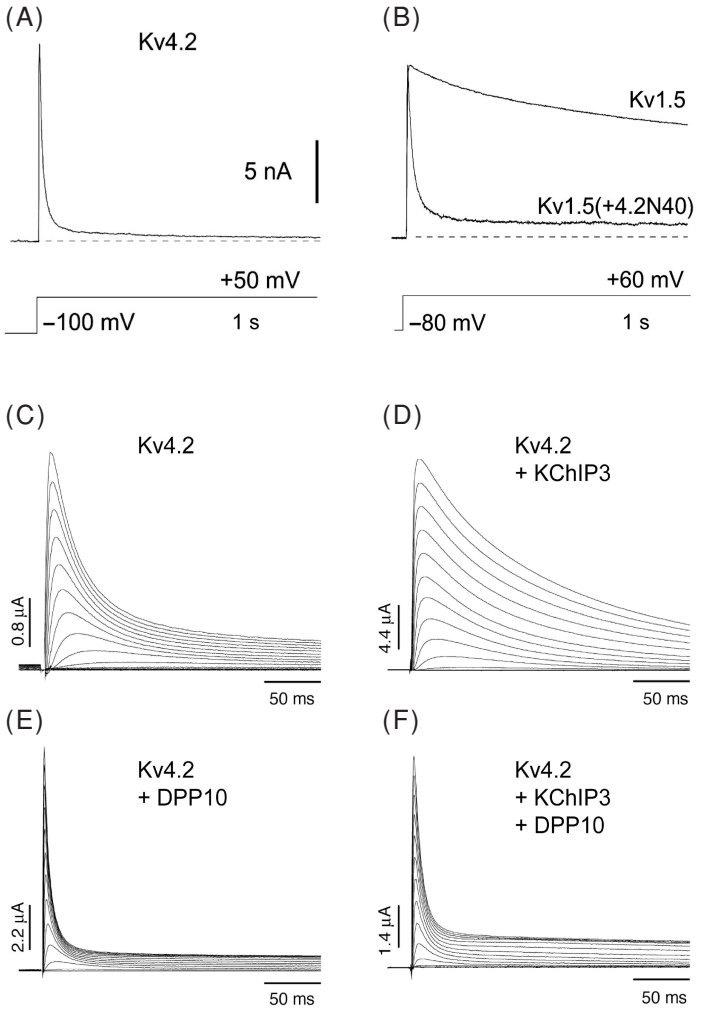

Fig. 4.8 Fast inactivation in Kv4.2 channels, and modification of Kv4.2 channel currents by KChIP3 and DPP10. (A) Illustration of a rapidly inactivating, Kv4.2 channel current. (B) Inactivation in Kv4 channel currents is complicated, potentially being caused by three or more different mechanisms. Elimination of the 40 terminal amino acids from the N-terminus of Kv4.2 slows inactivation, but still leaves a channel with a relatively fast inactivation process (see Gebauer et al., 2004; not shown). However, concatenation of the 40 N-terminal amino acids from Kv4.2 onto the slowly inactivating Kv1.5 imparts a fast inactivation mechanism onto Kv1.5. This demonstrates that the classical N-type ball and chain mechanism contributes to Kv4.2 channel inactivation. (C–D) Co-assembly with KChIP3 markedly slows Kv4.2 channel inactivation. (E) Co-assembly with DPP10 speeds Kv4.2 channel inactivation. (F) Co-assembly with both KChIP3 and DPP10 produces an intermediate inactivation rate. The rate of inactivation can be increased or decreased by changing the stoichiometry of expression of KChIP3 and DPP10 (Jerng et al., 2005). Currents in A–B were recorded in HEK cells, currents in C–F were recorded in CHO cells. Material for this figure was kindly provided by Robert Bahring (panels A–B), and Henry Jerng (panels C–F), and adapted from Gebauer et al., 2004 (panels A–B) and Jerng et al., 2005 (panels C–F).

dendrites, which results in larger epsps and action potentials in distal dendrites, reduces the threshold for LTP (Ramakers and Storm, 2002).

Reduction of Kv4.2 currents also occurs as a result of protein kinase-mediated phosphorylation of sites on the C-terminus (Anderson et al., 2000; Yuan et al., 2002). It appears that the channel is directly phosphorylated by MAP kinase, but channels can also be influenced indirectly by protein kinase A and protein kinase C, which activate MAP kinase (Yuan et al., 2002). Thus, there are many neurotransmitter-mediated pathways that can produce a reduction in I_A. As expected, this biochemically-mediated reduction of Kv4.2 current also leads to an increase in action potential amplitude in distal dendrites. Importantly, inhibition of MAP kinase reduces action potential boosting, inhibits the formation of LTP, and impairs learning (Watanabe et al., 2002; Morozov et al., 2003). Finally, Kv4.2 channels also localize Ca^{2+}-dependent events. For example, glutamate receptor activation, which would accompany excitatory synaptic input, triggers Ca^{2+}-mediated plateau potentials (Wei et al., 2001). The Ca^{2+} influx associated with these potentials would serve to activate myriad biochemical events, including second messenger cascades that modulate synaptic plasticity. With intact Kv4.2 channel function, these potentials are quickly terminated and therefore ~~remained~~ localized to the dendritic segment in which they were triggered (Wei et al., 2001; Cai et al., 2004). However, when Kv4.2 currents are inhibited, Ca^{2+} plateau potentials invade neighboring dendritic segments (Cai et al., 2004). In summary, depending on the role of a neuron within a circuit, dendritic membranes might contain members of the Kv1, Kv2, Kv3, Kv4, and/or K_{Ca} families (cf. Du et al., 1998; Mitterdorfer and Bean, 2002; Cai et al., 2004). Among them, Kv4.2 channels appear to be key players in the control of synaptic plasticity, and represent a common pathway by which either coincident electrophysiological stimuli or neurotransmitter-mediated biochemical cascades influence and localize events associated with neuronal plasticity and probably, learning and memory.

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4.14.2 The Kv4 Channel Complex

Channels composed of just the Kv4 α subunit do not display biophysical properties identical to I_A currents in neuronal membranes. Indeed, it appears that native Kv4 channels exist in a complex with members of at least two other protein families: one called Kv channel-interacting proteins (KChIPs), a member of a larger, Ca^{2+} binding protein family, and one called dipeptidyl peptidase-like proteins (DPLs; An et al., 2000; Nadal et al., 2003; Rhodes et al., 2004; Jerng et al., 2005). The KChiIP family of proteins are cytoplasmic, and bind to the N-terminus of the Kv4 channel (cf. Scannevin et al., 2004). DPLs are integral membrane proteins that belong to a serine protease family of proteins but are without catalytic activity (Qi et al., 2003). Complexing with either KChIPs or DPLs increases Kv4 surface expression (Shibata et al., 2003; Jerng et al., 2004b), but also produces important changes in biophysical behavior. Typically, complexing with KChIPs slows inactivation (Fig. 4.8; Beck et al., 2002), but depending on KChIP and α subunit species, it can also speed inactivation (Beck et al., 2002) or eliminate inactivation (Holmqvist et al.,

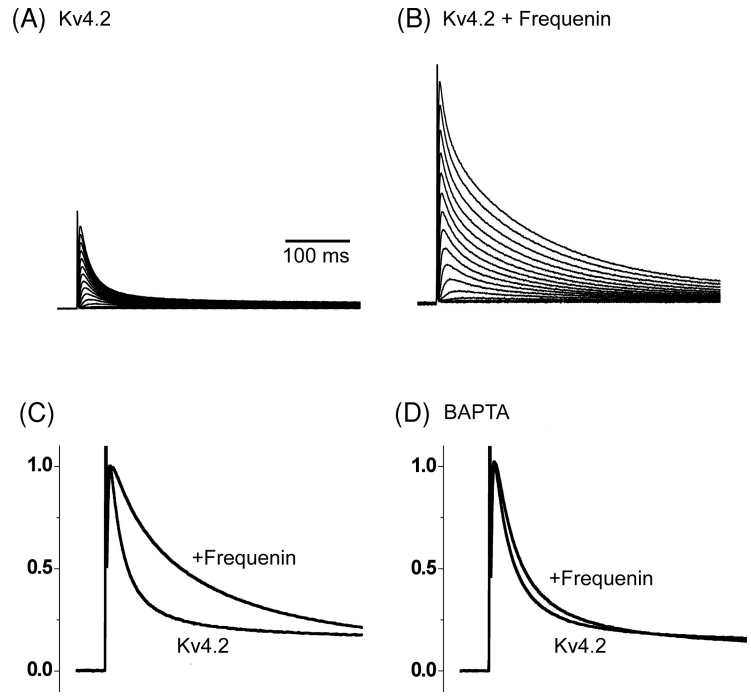

Fig. 4.9 Modulation of Kv4.2 channel currents by frequenin. (A–B) Co-expression of Kv4.2 channel subunits with frequenin, a Ca^{2+} binding protein similar to the KChIPs, increases channel expression and slows inactivation. (C) Normalized currents illustrate the slowing of Kv4.2 inactivation by co-assembly with frequenin. (D) With the Ca^{2+} chelator, BAPTA, in the cytoplasm, frequenin has little or no influence on Kv4.2 channel currents. This demonstrates that co-assembly with Ca^{2+} binding proteins can impart a Ca^{2+} dependence to Kv4.2 channel function. Currents were recorded from oocytes. Figure kindly provided by William A. Coetzee, adapted from Nakamura et al. (2001).

2002). Complexing of Kv4.2 with DPP10 speeds inactivation (Fig. 4.8; Jerng et al., 2005), and can also speed recovery from inactivation and the voltage dependence of activation and inactivation (Nadal et al., 2003). It appears that the complex formed by all three protein components represents the complete channel that underlies the I_A current (Nadal et al., 2003; Jerng et al., 2005). Moreover, it appears that varying the stoichiometry of the complex might be an approach used by cells to change I_A kinetics (Jerng et al., 2005).

The KChIP family of proteins may have an additional regulatory action on Kv4 channels. Frequenin is a member of the same Ca^{2+} binding protein family as the KChIPs. As with most other KChIP proteins, frequenin increases expression and slows inactivation of currents carried by Kv4.2 channels (Fig. 4.9A–B; Nakamura et al., 2001). However, the influence of frequenin on Kv4.2 currents is Ca^{2+}-dependent. If the rise in intracellular Ca^{2+} is prevented upon depolarization, frequenin has no impact on inactivation rate of currents carried by Kv4.2 channels

(Fig. 4.7C–D; Nakamura et al., 2001). Due to a structural difference in the N-terminal domain, frequenin influences only Kv4.2 and Kv4.3 channels; Kv4.1 channels are essentially unaffected (Nakamura et al., 2001).

In this section we presented only a small sampling of a massive and as yet incomplete literature on the role and regulation of Kv4 channels. However, we attempted in this sampling to provide insight into the enormous complexity of their involvement in neuronal physiology. Whereas Kv3 channels appear to have a rather more defined role in the accurate transmission of high-frequency input information into output information, Kv4 channels appear to have a more integrative role. Whether or not LTP, and perhaps learning and memory, occur depends on the readiness of Kv4 channels to respond to depolarization. Moreover, the size of Kv4 channel currents determine not only how large a local postsynaptic response will be but also how far it will spread down the dendrite. The sources of modulation of Kv4 current amplitude are manifold and intertwined: current amplitude is modulated by electrical activity, protein kinase activity, intracellular Ca^{2+} levels, and association with different protein partners. As a result of the fast inactivation mechanism, and the voltage range over which they are activated, Kv4 channel readiness can be modulated by changes in voltage dependence of activation or inactivation, the rate of inactivation or the rate of recovery from inactivation. Indeed, modulators not only influence one or more of all of these biophysical properties, but a number of modulators also influence cell surface expression levels. Thus, these modulators likely regulate long-term as well as short-term changes in Kv4 current amplitude.

Acknowledgments

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