**Structure and Function of Kv4-Family Transient Potassium Channels**

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Birnbaum, Shari G., Andrew W. Varga, Li-Lian Yuan, Anne E. Anderson, J. David Sweatt, and Laura A. Schrader. Structure and Function of Kv4-Family Transient Potassium Channels. *Physiol Rev* 84: 803–833, 2004; 10.1152/physrev.00039.2003.—Shal-type (Kv4.x) K⁺ channels are expressed in a variety of tissue, with particularly high levels in the brain and heart. These channels are the primary subunits that contribute to transient, voltage-dependent K⁺ currents in the nervous system (A currents) and the heart (transient outward current). Recent studies have revealed an enormous degree of complexity in the regulation of these channels. In this review, we describe the surprisingly large number of ancillary subunits and scaffolding proteins that can interact with the primary subunits, resulting in alterations in channel trafficking and kinetic properties. Furthermore, we discuss posttranslational modification of Kv4.x channel function with an emphasis on the role of kinase modulation of these channels in regulating membrane properties. This concept is especially intriguing...
as Kv4.2 channels may integrate a variety of intracellular signaling cascades into a coordinated output that dynamically modulates membrane excitability. Finally, the pathophysiology that may arise from dysregulation of these channels is also reviewed.

I. INTRODUCTION

This review focuses on the shal-type (Kv4.x) primary subunits of K⁺ channels and what we have learned about their function and regulation over the past few years. In our view, this is a timely topic and an emerging area of great physiological importance. A number of important and exciting new papers, primarily concerning studies of the nervous and cardiovascular systems, have been published in this area recently. This has prompted us to bring together an overview of this rapidly progressing area. One emerging theme from the recent literature is that K⁺ channels operate as supramolecular complexes, comprised of pore-forming primary, or α, subunits plus a number of associated ancillary subunits and scaffolding proteins. Until recently these proteins, which interact with the primary subunits, were unknown, and their discovery has revised our consideration of shal-type K⁺ channel function. This viewpoint will be one of the unifying themes of this review in which we will specifically discuss proteins that have recently been shown to interact with Kv4.x subunits.

A second theme we will emphasize is the complexity of the mechanisms mediating dynamic regulation of the channel. In particular, the structure-function relationships for posttranslational modification of Kv4.x channels are beginning to be understood in much greater detail. These mechanisms will be discussed in the context of the physiological role of Kv4.x channels in modulating membrane excitability, particularly in the heart and in neurons. Much work has focused on kinase regulation of A-type K⁺ currents in neurons and direct phosphorylation of the Kv4.2 channel subunit. Regulation of Kv4.2 channels and the A-type K⁺ currents that they mediate in hippocampal dendrites will be discussed in particular detail. An especially intriguing aspect of these types of regulation is the possibility that Kv4.2 channels serve as molecular signal integration devices, allowing the cell to integrate a variety of cell-surface signals into a coordinated output in terms of membrane electrical properties.

A. K⁺ Channel Overview

Three groups of K⁺ channels have been characterized based on putative membrane topology of their principal subunits. In all three cases the primary subunits tetramerize to form a single transmembrane pore (reviewed in Ref. 42). The first group, typified by voltage-activated and Ca²⁺-activated K⁺ channels, has six transmembrane domains per α-subunit. The second group, typified by the “leak” K⁺ channels, has four transmembrane domains in their α-subunits. Finally, the “inward rectifiers” are the simplest structurally and have two transmembrane domains in each α-subunit. Each of these three groups comprises a discrete family, based on sequence homology, which is further divided into subfamilies. Thus, based on nucleotide sequence analysis, it is obvious that many different K⁺ channels with diverse kinetics and functions exist. Diversity is also increased by their ability to form functional heterotetrameric structures and to associate with auxiliary or β-subunits.

The Shaker channel was the first K⁺ channel to be cloned. It is a voltage-dependent channel that was identified in a hyperexcitable Drosophila mutant (99, 147, 153). Other genes from Drosophila have been identified that bear ~40% sequence homology with Shaker channels, and thus are included in the Shaker superfamily. These include the Shab, Shave, and Shal subfamilies (179). The Kv channels are the mammalian gene counterparts for these Drosophila genes and bear 50–75% homology to the Drosophila genes. A systematic nomenclature based on amino acid sequence of the α-subunits has been developed that defines the Shaker subfamily as Kv1.x, the Shab subfamily as Kv2.x, the Shave subfamily as Kv3.x, and the Shal subfamily as Kv4.x. All these channels are gated by transmembrane voltage, hence the Kᵣ nomenclature.

The Shal-type family in mammals is comprised of three distinct genes: Kv4.1, Kv4.2, and Kv4.3. The proteins encoded by these genes are highly homologous within the transmembrane regions, with divergent amino and carboxy termini. Kv4.x family channels in general are highly expressed in the brain, heart, and smooth muscles. Heterologous expression of these proteins in expression systems demonstrates that they activate at subthreshold membrane potentials, inactivate rapidly, and recover from inactivation quickly compared with other Kv channels (See Fig. 1A). Therefore, they are termed transient currents. Recent data from transgenic and knockout animals as well as expression of dominant negative constructs indicate that these channels participate in the transient outward A-type K⁺ current characterized in the somatodendritic compartments of neurons, as well as form the Ca²⁺-independent A-type K⁺ current (transient outward current or Iₒ) in cardiac myocytes. The A-type K⁺ current in these specific tissue systems is discussed in great detail in sections VI and VII. It is important for us to note that Kv4.x are not the only primary subunits that form A-type K⁺ currents; Kv1.x primary subunits also are...
capable of forming A-type K\(^+\) currents, but are not discussed in great detail in this review. Finally, molecular approaches such as RNA interference, antisense knockdown, dominant negative constructs, and genetically engineered mice are becoming increasingly utilized to define roles for Kv4.x channels.

In addition to kinetics, Kv4.x channels can be identified based on pharmacology. Kv4.x channels, like many other K\(^+\) channels, are sensitive to 4-aminopyridine. More specific blockers of the Kv4.x family have recently been described. For example, the heteropodatoxins (167) are specific for the \(I_{h}\) of cardiac myocytes (29, 220), a cellular current likely composed at least in part of Kv4.x family channels. More recently, the phrixotoxins were characterized (49) and are also specific blockers of Kv4.x channels as well as the \(I_{to}\) in the heart.

The kinetic properties of the transient A-type K\(^+\) current differ greatly depending on the type of cell in which the current is expressed. This can depend on heteromultimerization between primary subunits when a cell
expresses more than one Kv4.x subtype primary subunit (e.g., Kv4.2 multimerizes with 4.3 in rat ventricular myocytes; see sect. vi), interaction with various other interacting proteins expressed selectively in various cells (see sect. iv), or posttranslational modification of the channels (see sect. v). We illustrate this diversity in A-type K+ currents, even within a single cell, by drawing your attention to Figure 1. This figure illustrates a current-membrane voltage plot from recordings at two different sites on the dendrite of a hippocampal pyramidal neuron. Notice that the activation curve is shifted 10 mV in the hyperpolarized direction for currents that are >100 μm away from the cell soma versus the currents recorded in more proximal dendritic regions. This indicates that the channels underlying the current in the distal dendrite open at a lower membrane potential than those in the proximal dendrites. This likely indicates differing primary or interacting subunits in Kv4.x-encoded channels in proximal dendrites compared with distal dendritic regions in these cells. However, we also know that the voltage dependence of activation of the distal currents is modulated by protein kinases, which is discussed in section v (76, 77, 228), so the different properties within different dendritic regions could be the result of differing posttranslational modification. Regardless of the underlying molecular mechanism, these findings clearly indicate subdomain specificity in the biophysical properties of A-type K+ channels.

Further evidence of cell context-dependent A-current properties is provided by considering the biophysical properties of the pore-forming Kv4.x α-subunit expressed in heterologous systems versus the properties of native Kv4.x-encoded channel in the cell membrane. This also is illustrated in Figure 1. Consider that the A-type K+ current in hippocampal dendrites (Fig. 1B) is composed of, at least in part, Kv4.2 subunits. The activation and inactivation curve recorded from Xenopus oocytes expressing only Kv4.2 is shown in Figure 1C. Kv4.2 inactivation and activation curves in the oocyte expression system are hyperpolarized relative to the hippocampal A-type K+ current, particularly the current in the more proximal dendrites. This suggests that less depolarization is necessary to open the channels and fewer channels are inactivated at a given membrane potential in the oocyte versus dendrite. In addition, the Kv4.2 currents in oocytes inactivate slower and recover from inactivation much more slowly than the native current (not shown). Therefore, the Kv4.2 subunits must be modified in the hippocampal pyramidal neuron relative to the isolated α-subunit expressed in oocytes, possibly by the effects of interacting subunits as well as by phosphorylation. We discuss specific possible mechanisms for these effects in later sections of the review.

II. MOLECULAR STRUCTURE

Analysis of various K+ channel sequences indicates several structural elements that are present in most K+ channels (see Fig. 2). These include the amino-terminal cytoplasmic domain, the T1 assembly domain, the six transmembrane α-helical domains (S1-S6); the voltage sensor (S4), the pore domain (P-loop), and a carboxy-terminal cytoplasmic domain.

Voltage-gated K+ channels exhibit a great amount of homogeneity within the transmembrane and pore-forming domains. It is now clear that K+ channels are composed of a tetramer of the primary subunits, where four primary subunits form the infrastructure of the channel with symmetry around the central pore. These K+ channel primary subunits can assemble as homo- or heteromultimers (89, 164). In this section we discuss the various functional and structural elements of K+ channels in general and Kv4.x channels specifically, focusing on mechanisms of voltage-sensing, mechanisms of inactivation, and the structural basis of subunit tetramerization.

A. Mechanisms of Voltage-Sensing

A detailed review of the mechanisms of voltage-sensing is beyond the scope of this review, but we will superficially discuss several recent, exciting papers that have sparked a debate in this area. We will review what is known about K+ channel voltage-sensing from known crystal structures to date. These data are derived from the bacterial K+ channels, whose pore-forming region exhibits homology with known mammalian K+ channels including the Kv4.x channels.

The membrane-spanning domain of all voltage-dependent K+ channels contains two highly conserved portions, the voltage-sensing portion that surrounds the central pore and the pore domain itself. The pore domain, S5, the P-loop, and S6 all together make up the ion permeation pathway, including the selectivity filter (Fig. 2A). A large body of evidence suggests that the voltage-sensing region of voltage-gated K+ channels is the fourth transmembrane α-helix, or S4. This region of the protein contains positively charged arginine or lysine residues at essentially every third position and is the only transmembrane domain that is appreciably charged (82). Membrane depolarization causes a movement of the positively charged residues of S4 through the gating canal. This movement of charges through the electric field of the membrane mediates the actual opening of the channel and generates what is referred to as a gating current.

Most data on the pore domains and voltage-sensing mechanisms come from the crystal structures of the bacterial channels, KcsA (52) and MthK (94), which show a high degree of homology with voltage-gated K+ channels.
Previous studies suggested that the S4 segment lies perpendicular to the plane of the membrane and is shielded by other parts of the channel from direct contact with the lipid environment. Upon membrane depolarization, the S4 segment transfers charged side chains between locations on opposite sides of the membrane, generating a gating current (24). Measurements of the gating charge moving across the membrane suggest that approximately four charges per subunit move entirely across the membrane during the channel’s activation by depolarization (5). Mutagenesis studies in which these charges are neutralized reduce the gating charge measured with activation (5, 175). In addition, an acidic residue in S2 was also found to contribute to gating charge of Shaker channels (175).

Independent groups have found that the S4 helix responds to voltage with rotational motion (37, 64) using LRET and FRET imaging, respectively. In addition, a similar rotational motion was found using histidine scanning to study changes in exposure of basic residues (190, 191). This rotational motion is likely coupled with translational motion to ultimately give rise to a “helical screw” type of motion (62).

This view has been somewhat challenged by the recent X-ray structure of another bacterial voltage-gated K⁺ channel, KvAP (95, 96), which interestingly contains an intracellular S4 domain. An examination of the voltage-sensing region of this channel suggests that this domain lies in a reclining position at the periphery of the channel, nearly in the plane of the membrane, in the closed state. Upon activation, it is proposed that the S4 domain moves through the hydrophobic lipid environment in a rowing, or paddlelike, motion to a more perpendicular position. The approach taken in these experiments, while innovative, may however introduce problems in interpretation of the data. These studies used detergent solubilization and formation of a complex of the ion channel with a monoclonal Fab fragment, which might have altered the gating mechanism. In this vein, Laine et al. (111) recently published evidence against the paddlelike movement using the MthK channel. Their experiments indicate that the S4 region actually lies in close proximity to the pore domain and that it interacts directly with the pore domain upon membrane depolarization. These results are not consistent with the paddle model of voltage-sensing. Thus the precise structural basis for voltage-sensing is not completely clear at present. Although all current models invoke the S4 domain as a voltage sensor, the details of how this works awaits further investigation.

B. Mechanisms of Inactivation

Channels formed from Kv4.x subunits rapidly inactivate. In general, two potential mechanisms for the inactivation of voltage-gated ion channels are well characterized. Both mechanisms apply to Kv4.x-encoded channels, although they have been more extensively studied in Shaker family channels. One mechanism of inactivation is
termed N-type or “ball and chain” type inactivation. This mechanism involves the tethered amino-terminal inactivation domain (ball) of the channel (230) or a similar domain in a β-subunit (158), binding to the intracellular entrance of the pore (90). This type of inactivation occurs only when the channel is already open, has fast kinetics, and can be eliminated by removal of the amino terminus (83, 84). A second type of inactivation is known as C-type inactivation and involves the pinching of the pore near the selectivity filter, resulting from a structural change in the four subunits (119, 142, 146). This type of inactivation is typically slower than N-type inactivation and can occur even in the absence of the amino-terminal domain of the channel (84). C-type inactivation is slower in the absence of N-type inactivation, suggesting that the two mechanisms are coupled (21, 84).

However, evidence suggests that Kv4.x family members, and Kv4.2 in particular, may not manifest these inactivation mechanisms in the classical sense. For example, deletion of the first 40 amino acids from the amino terminal of Kv4.2 results in a slowing of the fast and intermediate components of inactivation (15), rather than the complete loss of the fast component observed with amino-terminal deletion of Shaker channels. Kv4.1, on the other hand, loses the fast component of inactivation when the amino terminus is deleted (92, 93). Moreover, a positively charged domain at the carboxy terminus of Kv4.1 (amino acids 420–550) is necessary for rapid inactivation. Therefore, it appears that both the amino and carboxy termini of Kv4.1 are involved in inactivation gating (92, 93). Furthermore, several criteria for C-type inactivation, including interference by external tetraethylammonium (40), and slowing by high external potassium concentrations (21), are not found in Kv4.1- or Kv4.2-encoded channels (15, 17, 92). One key difference between Kv4.2 and Shaker channels that may account for this difference is that Kv4.2 channels predominantly inactivate from the closed state and recover directly, bypassing the open state (15), whereas Shaker channels inactivate only from the open state (45). One report, however, does suggest that Kv4.3 exhibits C-type inactivation (54). Thus subtle structural differences may account for the different attributes of channel inactivation manifest by Kv4.x versus Shaker subfamily channels.

C. The T1 Domain and K+ Channel Multimerization

Voltage-gated K+ channels only multimerize with members of their own subfamily. For example, mammalian members of the Kv4.x subfamily can multimerize and form functional channels with Kv4.x members from invertebrates, but they will not multimerize with mammalian members of the Kv1–3 subfamilies. The structural feature that mediates this is a highly conserved, cytoplasmic amino-terminal portion of the channel known as the tetramerization domain, or T1 domain (180). The T1 domain consists of ~130 amino acids directly preceding the first transmembrane domain, and on its own, can form a stable tetramer in solution. The crystal structure of the T1 domain suggests that the specificity of subfamily associations are based on the polar interfaces between subunits (106). While the primary function of the T1 domain is in channel tetramerization, it also appears to play a role in channel gating. Mutations within this region can produce either rightward or leftward shifts in the activation curve and either a speeding or slowing of inactivation depending on the particular mutation (44).

III. SUBCELLULAR LOCALIZATION AND TRAFFICKING

Specific localization and trafficking mechanisms are relevant to Kv4.x channel function in most cells where they have been studied. This is particularly relevant in cells with a complex morphology and many distinct subcellular compartments, such as neurons. In this section we discuss mechanisms for general channel trafficking and for the localization of Kv4.x channels to specific subcellular compartments. One exciting area of recent research is investigating the role of Kv4.x ancillary subunits in channel expression and trafficking. We will highlight recent findings from these studies in the following section as well.

One factor necessary to understand the role of Kv4.x channels in neuronal function is to understand their specialized distribution within a cell. For example, both Kv4.2 and Kv1.4 channels mediate a transient, A-type K+ current; however, Kv4.2 is localized to the soma and dendrites of neurons, while Kv1.4 is concentrated in axons (181). Intrinsic structural features of Kv4.x channels may regulate the subcellular trafficking of these channels. A recent study has shown that this subcellular targeting is mediated at least in part by a 16-amino acid dileucine-containing motif (160) within the pore-forming α-subunit. A comparison of all known mammalian and invertebrate Kv4.x channels reveals that 13 of the 16 amino acids in this sequence motif are conserved. Deletion of these amino acids (474–489) from Kv4.2, or merely replacing the two leucines with an alanine and valine, disrupts the polarization normally seen with Kv4.2. Instead of being targeted to the cell body and entire dendrite, distribution is limited to the proximal dendrite and proximal axon. Conversely, insertion of the 16-amino acid dileucine motif into the carboxy terminus of Kv1.3 and Kv1.4 is sufficient to alter their subcellular localization from axons to dendritic processes.

In cardiac ventricular myocytes, Kv4.2 has been shown to be concentrated in the sarcolemma (plasma
membrane, Ref. 19), where the highest level of immuno-
reactivity is found at the intercalated disk region that
connects myocytes together. Using high-resolution tech-
niques, Takeuchi et al. (192) have shown that Kv4.2 local-
izes to the sarcolemma in atrial myocytes; however, in
ventricular myocytes Kv4.2 is predominant in the t tub-
ules rather than the peripheral membrane. Thus Kv4.2 is
selectively targeted to specific subcellular domains in
cardiac myocytes as well. However, the mechanism that
targets Kv4.x channels in myocytes has not yet been
investigated.

Another factor necessary for regulating channel lo-
calization is release from the endoplasmic reticulum
(ER). Several intrinsic sequences that regulate retention
of proteins in the ER or export of the protein from the ER
have been identified (120). One important signal for the
trafficking of membrane channel proteins in general is the
RXR ER retention motif. This has been shown to be
necessary for ER retention in both KATP channels (231)
and the NR1 subunit of N-methyl-D-aspartate (NMDA)
receptors (173). Although an RXR ER retention signal has
not been specifically identified in Kv4.2, there exists an
RKR in its amino terminus, an RYR in an intracellular loop
between the second and third transmembrane domains,
and an RIR in its carboxy terminus, any of which would
serve as a good candidate for ER retention. Mutation of
the amino-terminal RKR potential ER retention signal did
not alter the ER localization of Kv4.2 in COS cells (183),
suggesting that this particular site is not involved in ER
retention; however, other potential ER retention sites in
Kv4.2 have not been examined. Furthermore, ER export
signals, which can accelerate the ER export of proteins,
are now being identified (reviewed in Ref. 120). This is
another area that has not been closely examined for Kv4.x
channels.

Channel trafficking from the ER may also be regu-
lated by interactions with auxiliary proteins as well as
potentially by channel phosphorylation (reviewed in Ref.
120). For example, coexpression of a PDZ domain-con-
taining protein, SAP-97, with the Kv1 family of K+ chan-
nels inhibits ER release of the channel and dramatically
reduces their cell surface expression (195). Protein phos-
phorylation has also been shown to enhance ER export
for NMDA receptor subunits (173). As Kv4.x channels can
be phosphorylated by several kinases (see sect. v), this is
another potential mechanism for regulating channel sur-
face expression. Overall, much more work is required to
understand the targeting and distribution of Kv4.x chan-
nels; however, these studies suggest that altered release
from the ER may regulate surface expression of Kv4.x chan-
nels, and ultimately changes in cellular excitability.

A large variety of proteins that interact with Kv4.x
channels have now been identified (see Table 1). Many
of these auxiliary proteins appear to play a role in the
localization of Kv4.x channels within the cell. Further-
more, many of these interacting proteins have also been
shown to alter the biophysical properties of Kv4.x chan-
nels. The effects of these interacting proteins on
Kv4.x channel kinetics and cellular distribution are
discussed in section iv.

IV. INTERACTING SUBUNITS

A. Kvβ

Functional diversity of K+ channels can be aug-
mented through the association of the pore-forming
α-subunits with auxiliary subunits. We now know of many
auxiliary subunits that interact with α-subunits and con-
tribute to the regulation of biophysical properties and
expression levels of K+ channels. The auxiliary subunits
discovered first and characterized in most detail are the
members of the Kvβ auxiliary subunit family.

Kvβ subunits lack putative transmembrane domains
and potential glycosylation sites or leader sequences, sug-
gesting that they are cytoplasmic proteins (174). Three
Kvβ genes have been identified: Kvβ1, Kvβ2, and Kvβ3
(73, 113). Several functional consequences of β-subunits
on Kv channels have been described. One effect is to
increase the inactivation rate of Kv channels (73). In a
striking example, Kvβ subunits can convert normally non-
inactivating delayed rectifier channels to a rapidly inacti-
vating channel (113, 158). Kvβ1 and Kvβ2 have also been

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**TABLE 1.** *Kv4.2 interacting proteins*

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<tr>
<th>Interacting Protein</th>
<th>Interacting Domain on Kv4.2</th>
<th>Possible Function</th>
<th>Reference Nos.</th>
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| KChPs 1, 2, 3, and 4| Amino-terminal cytoplasmic domain              | Trafficking, biophysical properties, PKA and AA modula-
|                     |                                                 | tion                                                   | 16–19          |
| Kvβ subunits        | Carboxy-terminal cytoplasmic domain            | Oxygen sensitivity, expression levels                  | 20             |
| PSD-95              | Carboxy-terminal 4 amino acids                 | Surface expression, clustering                         | 13             |
| Filamin             | Amino acids 600–604 and adjacent region        | Expression levels, cytoskeletal interactions           | 10             |
| Frequenin (NCS-1)   | Amino-terminal cytoplasmic domain              | Expression levels, recovery from inactivation          | 11             |
| minK-related peptide| Unknown                                         | Biophysical properties                                 | 12             |
| DPPX                | Unknown                                         | Expression levels, biophysical properties               |                |

PKA, protein kinase A; AA, arachidonic acid.

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shown to modulate voltage dependence of Kv channels in heterologous expression systems (56). Another function of β-subunits is to serve as chaperone proteins that promote and/or stabilize cell surface expression of K⁺ channel α-subunits in general (57, 116, 135, 136, 182, 199), including Kv4.3 channels (224). Another proposed role for Kvβ subunits is as redox sensors, as they structurally are similar to oxidoreductase enzymes (67) and may confer redox sensitivity to channel function through a bound NADPH cofactor.

The effects of β-subunits specifically on Kv4.x channel function is still under investigation. While Kvβ1.2 does not affect Kv4.2 channel gating kinetics, it does confer sensitivity to redox modulation and hypoxia (149). Kv4.3 interacts with all three known β-subunits (β1, β2, and β3, see Refs. 48, 224). Kvβ1 and -2 cause an increase in Kv4.3 channel current density, with no effect on channel gating (224), while β3 shifts steady-state inactivation and slows recovery from inactivation. In addition, Kvβ1 and -2 subunits were found to localize in many of the same brain structures as Kv4.x-family α-subunits, particularly the hippocampus (159), suggesting they are an integral component of neuronal A-type K⁺ currents.

B. K⁺ Channel Interacting Proteins

Although Kv4.x channels are the best candidates for mediating A-type K⁺ currents in neurons, Kv4.2 homomers expressed in nonneuronal systems manifest activation curves that are somewhat shifted in the depolarizing direction, and their recovery from inactivation is slower than what is observed for native A-type K⁺ currents. Interestingly, in early studies Serodio et al. (176) found that rat brain mRNA coexpressed with Kv4.2 in oocytes could restore many of the properties seen in native A-type K⁺ currents, suggesting that some cofactor was responsible for this change. A good candidate for this cofactor came to light in the form of a family of auxiliary proteins known as K⁺ channel interacting proteins, or KChIPs (11). Four KChIPs have been identified to date. KChIPs 1–3 were originally identified in a yeast-two-hybrid assay using the amino terminus of Kv4.3 as bait (11). KChIP4 was identified later as a binding partner of presephin 2 (131). The KChIPs are members of the recoverin-neuronal calcium sensor superfamly that are Ca²⁺-binding proteins. KChIPs share sequence similarity with neuronal calcium sensor 1 (NCS-1) or frequenin (discussed below), a calcium-binding protein that is involved in the regulation of transmitter release in Drosophila (154). Moreover, KChIP3 is identical to calnexin (33) and DREAM, a Ca²⁺-regulated transcriptional repressor (35). The various KChIPs have a variable amino-terminal region, but a conserved carboxy-terminal region that contains four EF-hand-like calcium-binding motifs. The function of these Ca²⁺ binding domains in K⁺ channel regulation is unknown; however, a Ca²⁺ dependence of transient K⁺ currents has been reported (28, 59, 188).

The KChIPs colocalize and coimmunoprecipitate with brain Kv4.x subunits through binding to the amino terminus of Kv4.x α-subunits and are thus considered integral components of the native Kv4.x channel complexes (11, 16). KChIP2 has also been shown to be an integral subunit in the ventricular wall of the heart where its expression parallels the gradient in transient outward current Iₒ (47, 148, 161, 162).

KChIPs 1, 2, and 3 all have similar effects on the physiological properties of Kv4.x channels, these effects having been mostly studied in Kv4.2 channels specifically. The physiological effects of KChIPs include an increase in the density of Kv4.2 currents, a hyperpolarizing shift in the activation curve, an increase in the rate of recovery from inactivation, and a slowing of the time constant of inactivation. The variable amino-terminal region of the KChIPs is unnecessary for this modulation to occur. However, modulation fails to occur when mutations are introduced into the EF-hand region in the conserved carboxy terminus of KChIPs (11).

Coexpression of KChIP1, 2, or 3 together with Kv4.2 increases the peak current density ~10-fold, suggesting that the KChIPs may promote surface expression, or stabilize the expression of Kv4.x channel proteins at the surface (11). A recent study has shown that COS1 cells transfected with Kv4.2 alone exhibit a perinuclear pattern of immunofluorescent staining, which is typical for ER retained proteins (183), and no detectable surface staining (Fig. 3). Furthermore, double-labeling of these cells for Kv4.2 and the ER protein calnexin revealed an overlapping staining pattern, while double-labeling with a Golgi apparatus marker revealed no overlapping staining. Coexpression of Kv4.2 with KChIP1, 2, or 3 dramatically altered the subcellular distribution of Kv4.2 channels (Fig. 3). Kv4.2 staining in the cotransfected cells exhibited significant cell-surface staining, and the remaining intracellular Kv4.2 colocalized with the Golgi apparatus marker rather than the ER marker. In contrast, KChIP4 does not promote surface expression of Kv4.2, and in fact, KChIP4 may competitively antagonize binding of the other KChIPs to Kv4.2 (183). Furthermore, unlike the other KChIPs, KChIP4 eliminates fast inactivation of Kv4.x-encoded currents (79).

Because KChIPs have strong effects on the modulation of Kv4.x channels, they stand out as targets themselves for functional regulation. In fact, it has been demonstrated that the ability of arachidonic acid to modulate the peak amplitude and kinetics of Kv4.x-encoded current is dependent on the presence of KChIPs (80). Similarly,
the ability of protein kinase A (PKA) to modulate the activation curve and time constant of inactivation of Kv4.2 also required KChIPs (169), an effect we will return to in section VI.

Overall, the ability of KChIPs to restore more native-like properties to Kv4.x channels and their colocalization with Kv4.x channels in several systems suggests that KChIPs may be an important and integral part of the composition of A-type K⁺ channels in cells.

C. NCS-1

Interestingly, NCS-1 (also known as frequenin), a member of the EF-hand family of Ca²⁺ sensing proteins which includes KChIPs, is also expressed in mouse brain and coimmunoprecipitates with Kv4.2 and Kv4.3 (69, 138). In heterologous systems, NCS-1 coexpression with Kv4.x α-subunits increases the current density and slows the rate of inactivation of the Kv4.x current. In contrast to KChIPs, however, NCS-1 does not affect the voltage dependence of inactivation or rate of recovery from inactivation of the channel. The effects of NCS-1 on current density and rate of inactivation are Ca²⁺ dependent, as they are blocked by a membrane-permeable Ca²⁺ chelator. These results are not due to a nonselective effect of Ca²⁺ binding proteins on Kv4.x channels, as other closely related members of the Ca²⁺ binding family (VILIP1, hippocalcin, neurocalcin, and calmodulin) have no significant effect on the amplitude or time course of Kv4.2-encoded current (138).

In addition to altering the biophysical properties of Kv4.x channels, NCS-1 can also alter the cellular distribution of Kv4.x channels. In COS cells transfected with Kv4.2 cDNA, Kv4.2 proteins were observed predominantly in perinuclear regions (138). However, cotransfection of Kv4.2 and NCS-1 increased the Kv4.2 immunoreactivity in the outer margins of the cells. Similarly, Guo et al. (70) reported an increase in surface expression of Kv4.3 channels coexpressed with NCS-1 in HEK-93 cells, and a decrease in surface expression of the channel when NCS-1 levels were reduced with an antisense oligonucleotide. In accordance with the enhanced surface expression of the Kv4.x channels, both groups reported an increase in A-type K⁺ current when the Kv4.x channel was coexpressed with NCS-1. These data suggest that in addition to regulating the rate of current inactivation for Kv4.2 and Kv4.3, NCS-1 can also regulate A-type K⁺ current by enhancing the surface expression of these channels.

D. K⁺ Channel Accessory Protein

K⁺ channel accessory protein (KChAP) was first cloned from a rat cDNA library and is not yet described in other species (211). KChAP is a member of the protein inhibitor of activated signal transducer STAT3 gene family. KChAPs are known to interact with several different families of Kv channels, including Kv1.3, 2.1, 2.2, and 4.3 (110, 109). Coexpression of KChAPs with these Kv chan-
nals increases current expression, without an effect on current kinetics. This chaperone activity of KChAPs may be mediated indirectly through an interaction with Kvβ1.2 as expression of Kvβ1.2 inhibits the chaperone effects of KChAP on Kv4.3 (110). The effects of KChAP on Kv4.1 or 4.2 are currently unknown.

E. DPPX

Although association of KChIPs with Kv4.2 or Kv4.3 in heterologous expression systems can restore most of the properties of native A-type K⁺ currents, one shortcoming is that they slow the kinetics of inactivation, compared with the fast kinetics of inactivation of native A-type K⁺ currents. In recent studies Bernardo Rudy’s group (133) discovered that coexpression of high-molecular-weight mRNA isolated from rat cerebellum with Kv4.2 in oocytes resulted in an acceleration of the kinetics of inactivation. This effect was not eliminated by using KChIP antisense oligonucleotides (133), suggesting the existence of another Kv4.x auxiliary protein that the authors labeled K⁺ channel accelerating factor, or KAF. Later, Rudy’s group found that KAF is a transmembrane protein called DPPX (134).

DPPX had no previously known function, although it is related to the dipeptidyl aminopeptidase CD-26, which has a role in cell adhesion. Coexpression of DPPX and Kv4.2 had similar effects to coexpression of KChIPs and Kv4.2: an increase in surface expression, increased speed of recovery from inactivation, and a shift in inactivation voltage dependence. However, the time constant of inactivation of Kv4.2 was decreased with coexpression of DPPX, making the current more similar to native currents (134). DPPX had similar effects when coexpressed with Kv4.3, but not with other fast-inactivating K⁺ channels such as Kv1.4.

DPPX localizes to the somatodendritic compartments of neurons that are known to contain Kv4.2, such as pyramidal neurons of the hippocampus and striatal neurons, as well as to regions that contain Kv4.3, such as the Purkinje cells of the cerebellum. DPPX also localizes to areas known to contain both Kv4.2 and Kv4.3, such as granule cells of the cerebellum and dentate gyrus (134).

DPPX also strongly alters the cellular localization of Kv4.x channels (134). Coexpression of Kv4.2 and DPPX in CHO cells results in a 20-fold increase in surface expression of the channel as measured by a surface protein biotinylation assay. This dramatic effect was due to a 2-fold increase in total protein levels as well as a 10-fold increase in the surface-exposed channel protein. The increase in surface expression of Kv4.2 protein correlated with a 25-fold increase in A-type K⁺ current when Kv4.2 was coexpressed with DPPX in Xenopus oocytes. The striking alteration in cellular localization of Kv4.2 channels as well as the ability to restore a more native A-type K⁺ current suggests that DPPX is also an integral molecular component of Kv4 channels in cells where they are coexpressed.

F. Cytoskeletal Proteins

A search for proteins responsible for Kv4.2 localization to somatodendritic compartments of neurons (181) led to the discovery that Kv4.2 interacts with filamin, a member of the α-actinin/spectrin/dystrophin family of actin-binding proteins. Petrecca et al. (151) demonstrated an association between the carboxy terminal of Kv4.2 and filamin in neurons. These investigators also showed that Kv4.2 and filamin colocalize in cerebellum and cultured hippocampal neurons. Furthermore, Kv4.2 is expressed in a punctate pattern in neuronal dendrites that colocalizes with the synaptic marker synaptophysin. Transfection of Kv4.2 into a heterologous cell line lacking filamin resulted in a uniform expression pattern of the channel, while similar expression of Kv4.2 in a cell line that contains filamin resulted in Kv4.2 colocalizing with filamin at the roots of filopods (151). Transfection of a mutant form of Kv4.2 in which the filamin-binding region had been altered resulted in a uniform expression pattern similar to that observed in the cells without filamin. Although total Kv4.2 protein expression was the same, the whole cell Kv4.2 current density from Kv4.2 transfected cells was two- to threefold greater in the filamin-expressing cells than in cells without filamin. This difference was due to a higher density of Kv4.2 channels in the surface membrane rather than any changes in single-channel conductance. These data suggest that filamin may function as a scaffold protein that enhances Kv4.2 channel expression on the surface membrane. Furthermore, an interaction with filamin may target Kv4.2 to synapses, although further research is needed to confirm this.

Interestingly, the critical region of Kv4.2 interaction with filamin appears to be the proline-rich regions of 601–604 and is identical to a sequence in Kv4.3 that also binds to filamin (151). These Kv4.2 residues critical for filamin association are in the region of an ERK phosphorylation site (T602) discussed in section VI. We speculate that ERK phosphorylation at this site could interfere with the Kv4.2/filamin association and result in altered Kv4.2 localization.

Another protein family that may play a role in Kv4.x localization and distribution is the integrins. Interaction with these cell adhesion molecules may underlie the restricted cellular distribution of the Kv4.x-interacting protein filamin (31). A variety of studies indicate that filamin and integrin interact and are components of the neuromuscular junction, where β-integrin plays a role in the signaling events that lead to agrin-induced clustering of ACh receptors (127).
A possible interaction of Kv4.2 with integrins is supported by a recent study that showed that the extracellular matrix protein vitronectin affects the expression of Kv4.2 in hippocampal explants (205). Vitronectin is an extracellular ligand for transmembrane integrin proteins. In developing hippocampal pyramidal neurons, vitronectin exposure for 3–4 days was found to increase the peak current amplitude of A-type K⁺ current. Vitronectin treatment had no significant effect on the voltage dependence of activation or inactivation, or the kinetics of inactivation or recovery from inactivation of the A-type K⁺ current. Immunocytochemical analysis revealed that vitronectin increased the immunoreactivity of Kv4.2 proteins, but not Kv1.4 proteins, suggesting that the changes in A-type K⁺ current are mediated by Kv4.2. Furthermore, vitronectin had no effect on the amplitude of the sustained components of K⁺ currents in these hippocampal pyramidal neurons, indicating a specific effect on A-type K⁺ currents. These data are indicative of a functional interaction of Kv4.2 with integrins, at least in neurons. While this prospect is enticing and promising, more research is necessary to confirm such an interaction and determine its molecular basis.

G. Neuron-Specific Modulator: PSD-95

Another synapse-associated protein found to interact with Kv4.2 is the scaffolding protein PSD-95 (216). Using a surface biotinylation assay, Wong et al. (216) showed that coexpression of Kv4.2 with PSD-95 in CHO cells enhanced the surface expression of Kv4.2 ~2-fold. Deletion of the last four amino acids of Kv4.2’s sequence (VSAL), which constitute a putative PDZ-binding domain, or using a palmitoylation-deficient PSD-95 mutant which blocks Kv1.4 channel clustering (85) blocked this increase in surface expression of Kv4.2 without affecting total channel levels (216). These results were confirmed using a fluorescently tagged Kv4.2 protein to monitor channel protein distribution. In cells transfected with Kv4.2 alone, the majority of the fluorescence was located in an internal reticular network with some fluorescence at the outer cell margins. Coexpression of Kv4.2 and PSD-95 resulted in an increase in channel expression on the cell surface as well as the formation of clusters of Kv4.2 channels. Thus PSD-95 could be another protein responsible for recruiting Kv4.x channels to the cell surface.

H. Heart-Selective Modulator: MinK-Related Peptide 1

MinK-related peptide 1 (MiRP1) is a protein linked to maintaining electrical stability in the heart. Initial functional data suggested that MiRP1 associates with and modulates the function of HERG, which contributes to the fast component of the delayed rectifier current of heart (2). More recently KvLQT1, the slower component of the delayed rectifier (196), as well as Kv3.4 and KCNQ4 (1, 171) were found to interact with MiRP1, suggesting it is a rather promiscuous binding protein. MiRP1 has been found to affect Kv4.2 currents expressed in Xenopus oocytes, slowing the rates of activation and inactivation and shifting the voltage dependence of activation in the depolarizing direction (232). These data strongly suggest that MiRP1 may contribute to regulating Ito in the heart, although this has not been tested directly. MiRP1 has been undetected in the brain other than in the pituitary (217), so the relevance of MinK to neuronal A-type K⁺ current remains to be seen.

V. REGULATION BY POSTTRANSLATIONAL MODIFICATION

An enormous variety of posttranslational modification of proteins has been described, including glycosylation, methylation, acetylation, ubiquitination, attachment of fatty acids, covalent attachment of coenzymes, and phosphorylation. Posttranslational modification of proteins can play an important role in the proper folding, assembly, and trafficking of many membrane-associated proteins. The role of several common forms of posttranslational modification of channels will be discussed in this section: palmitoylation, glycosylation, and phosphorylation, specifically in the context of regulating Kv4.x channels.

A. Palmitoylation

To our knowledge, most forms of posttranslational modification have not been well studied for Kv4.x channels. However, a few studies have examined the role of posttranslational modification of subunits that interact with Kv4.x channels (see sect. iv). There is substantial evidence that attachment of palmitate, a long-chain fatty acid, to KChIPs is required for the plasma membrane localization of both ancillary subunits and the associated Kv4.x channel. For example, two “long” isoforms of KChIP2 have been identified which contain potential palmitoylation sites. These two isoforms show enhanced surface membrane expression compared with the shorter isoform when expressed in the absence of Kv4.3 (194). Furthermore, the longer two isoforms increased the surface membrane expression of Kv4.3 channels and produced larger increases in Kv4.3 current density compared with the shorter KChIP2 isoform. Mutation of the palmitoylation sites on the longer KChIP2 isoform reduced both the plasma membrane localization of Kv4.3 channels as well as the enhanced current observed with wild-type KChIP2. These data suggest that palmitoylation of KChIP
is an important mechanism for enhancing cell surface localization of Kv4.x channels.

Palmitoylation also appears to be important for trafficking of PSD-95 to postsynaptic densities and for the formation of ion channel clusters (55, 198). The palmitoylation of PSD-95 is also required for PSD-95-mediated surface expression of Kv4.2. Furthermore, clustering of the K\(^+\) channel Kv1.4 at postsynaptic densities requires palmitoylation of PSD-95. These data suggest that a similar mechanism may be required to cluster Kv4.x channels at the cell membrane in postsynaptic densities. Obviously, there is much more work required to understand the role of palmitoylation in regulating Kv4.x channel function, cellular localization, and stability/degradation.

B. Glycosylation

Glycosylation of proteins has been shown to promote proper protein folding in the ER as well as to alter protein transport and targeting (for review, see Ref. 74). In general, glycosylation of K\(^+\) channels is not required for surface expression, but does appear to increase surface expression of the channel by decreasing channel turnover and increasing channel stability. For example, blocking glycosylation of S haker-type K\(^+\) channels has been shown to dramatically decrease the stability and cell surface expression of the channel but not the folding (104) and assembly of functional channels, or their transport to the cell surface (168). Similar results have been shown for the HERG K\(^+\) channel (65).

Concerning Kv4.x-family channels specifically, none of the channels contains a consensus sequence for attachment of an N-linked oligosaccharide (N-X-S/T) on their extracellular surface. While there is no known consensus sequence for O-linked glycosylation, we are not aware of any studies that have determined that any of the Kv4.x channels are directly glycosylated. However, treatment of dissociated ventricular myocytes with a neuraminidase to remove sialic acid, a negatively charged sugar residue, decreases I\(_{\text{to}}\) (which is produced by Kv4.2 and Kv4.3 channels, see Ref. 202). Accordingly, an increase in the duration of action potentials was also observed in these cells. Removal of sialic acid did not block the channels from reaching the cell surface, nor did it block the formation of functional channels, but it did alter the voltage dependence of activation in addition to reducing I\(_{\text{to}}\) amplitude. These results were mimicked by expression of Kv4.3 in a sialylation-deficient heterologous cell line. The reduction of I\(_{\text{to}}\) suggests that removal of sialic acid reduces the number of K\(^+\) channels at the plasma surface, possibly through a faster channel turnover rate that leads to a decrease in Kv4.3 channel stability. While it is feasible that sialic acid residues on Kv4.3 directly modulate the channel, it is more likely that the effects observed in this study were due to deglycosylation of a Kv4.x interacting subunit (see sect. iv).

C. Phosphorylation

In the last few years there has been a great deal of evidence that A-type K\(^+\) currents in both neuronal and cardiac cells can be regulated by phosphorylation. Application of a phorbol ester, which activates protein kinase C (PKC), suppresses I\(_{\text{to}}\) in ventricular myocytes (13, 137). Similarly, recording from pyramidal cell dendrites in the hippocampus, Hoffman and Johnston (76) showed that activation of either PKA or PKC decreased the probability of A-type K\(^+\) channel opening. PKA and PKC activation also increased the amplitude of back-propagating action potentials in distal dendrites, consistent with a decrease in K\(^+\) channel current (see sect. vii). It has subsequently been shown that PKA and PKC modulation of the A-type K\(^+\) current in hippocampal neurons is mediated through the ERK/mitogen-activated protein kinase (MAPK) pathway (228). Finally, activation of PKC suppressed Kv4.2 or Kv4.3 current in Xenopus oocytes (137).

Kv4.2 contains a consensus sequence for phosphorylation by protein tyrosine kinase (PTK), and it has previously been shown that Kv1.2 channels can be inhibited by PKC activation of PTK phosphorylation (115). Thus Nakamura et al. (137) mutated the consensus sequence in Kv4.2 for phosphorylation by PTK to determine if this site mediated the PKC inhibition of the A-type K\(^+\) current. Modulation of this Kv4.2 mutant was similar to the wild-type; therefore, PKC inhibits the Kv4.2 currents independent of direct PTK phosphorylation. Furthermore, Kv4.3, which is also inhibited by PKC, does not contain a consensus sequence for PTK phosphorylation.

Finally, it is worth noting that splice variants may potentially exhibit differential sensitivity to phosphorylation. For example, two splice variants of human Kv4.3 have been identified; the longer splice variant contains 19 amino acids with a consensus PKC phosphorylation site inserted into the carboxy terminus after the last transmembrane spanning region (105). PKC activation reduced the peak current in the longer splice variant but had no effect on the shorter splice variant when expressed in a heterologous expression system (152). Mutation of the PKC consensus site abolished the sensitivity of the long splice variant of Kv4.3 to PKC activation, suggesting that direct phosphorylation of Kv4.3 in the alternatively spliced domain modulated the K\(^+\) current.

As discussed above, phosphorylation of Kv4.2 by PKA has been shown to modulate the K\(^+\) current in hippocampal neurons (76). Surprisingly, PKA has no effect on K\(^+\) current when Kv4.2 is expressed alone in Xenopus oocytes (169). Further studies revealed that coexpression of KChIP3 with Kv4.2 was sufficient to rescue
the PKA regulation of Kv4.2 (see Fig. 4). Using mutations of the PKA phosphorylation sites in Kv4.2, Schrader et al. (169) found that both PKA phosphorylation of Kv4.2 at serine-552 in addition to an interaction between Kv4.2 and the ancillary subunit KChIP3 were required for PKA regulation of the A-type K⁺ current in oocytes. This finding leads to a completely unexpected conclusion—direct phosphorylation of the Kv4.2 α-subunit by PKA is not sufficient to modulate channel function; the effect of phosphorylation requires the presence of the KChIP ancillary subunit.

As an aside, the finding that PKA activation causes channel modulation in the oocyte system is in apparent contradiction to the finding that PKA regulation of K⁺ current in hippocampal neurons is completely blocked by the MEK inhibitor UO126 (228). However, it is likely that Kv4.2 is also phosphorylated by ERK in the oocyte system, and perhaps ERK phosphorylation of Kv4.2 is also required for PKA modulation of the K⁺ current. Another possibility is that Kv4.2 forms a larger supermolecular complex with other KChIP or interacting proteins in hippocampal neurons. The association with the other proteins may functionally mask the effect of PKA phosphorylation of Kv4.2. The interaction between Kv4.x channels and phosphorylation by PKA is further complicated by recent data from Shibata et al. (183) suggesting that Kv4.2 must be expressed at the plasma cell membrane (potentially through interaction with KChIPs) to be phosphorylated at serine-552. Although much more research is needed to fully understand the role of ancillary subunits in phosphorylation-dependent regulation of Kv4.x channels, these recent findings indicate an unexpected complexity to regulation of Kv4.x channels through phosphorylation.

Examination of the amino acid sequence for Kv4.2 and Kv4.3 reveals several consensus sequences for phosphorylation by PKA, PKC, ERK/MAPK, and calmodulin kinase II (CaMKII). Thus far, direct biochemical studies of Kv4.x channel phosphorylation have only been published for the ERK and PKA sites of Kv4.2. Two sites on Kv4.2 that are phosphorylated in vitro and in vivo by PKA have been identified (12) as well as three ERK/MAPK phosphorylation sites (3). However, the only functional study of the specific Kv4.2 phosphorylation sites that has been published concerns the serine-552 site, which mediates PKA modulation of Kv4.2 voltage dependence of activation when associated with KChIP (169). In addition, mutation of the other PKA sites on the amino terminal had no effect on channel kinetics.

As part of the biochemical studies of PKA and ERK phosphorylation of Kv4.2, three antibodies have been developed which selectively recognize Kv4.2 when it is phosphorylated at different sites: 1) when it is phosphorylated by PKA at the amino-terminal site (threonine-38), 2) when it is phosphorylated by PKA at the carboxy-terminal site (serine-552, see Ref. 12), and 3) when it is triply phosphorylated at all three ERK sites (threonine-602, threonine-607, and serine-616; see Ref. 3). With the use of these three antibodies, the distribution of phosphorylated Kv4.2 was examined in the mouse brain. Immunoreactivity for all three antibodies was found in many widespread regions including the cerebral cortex, hippocampus, thalamus, cerebellum, striatum, and amygdala (204). While immunoreactivity with each antibody was found in the same structures, the distribution of staining varied between the antibodies (Fig. 5). Of particular interest is the pattern of staining in the hippocampus. For example, the stratum lacunosum moleculare, which receives inputs from the entorhinal cortex via the perforant pathway, displays relatively little ERK-phosphorylated Kv4.2 or PKA carboxy-terminal-phosphorylated Kv4.2. However, this same layer is stained by the antibody that recognizes Kv4.2 when it is phosphorylated by PKA at the amino terminus. Similarly, of the three antibodies tested, the soma of CA3 neurons are primarily recognized by the ERK triply phosphorylated Kv4.2 antibody, and the mossy fiber inputs to CA3 are primarily recognized by the carboxy-terminal PKA-phosphorylated Kv4.2 (see Table 2 for

![FIG. 4. KChIP3 coexpression is required for modulation of the current by PKA. Activation curve of current obtained from oocytes transfected with either Kv4.2 alone (A) or Kv4.2 + KChIP3 (B) is shown. The current was evoked from depolarizing pulses in control (squares) and 15 min after commencement of forskolin application (triangles). Forskolin does not significantly alter recovery from inactivation (not shown). [Adapted from Schrader et al. (169).]](http://physrev.physiology.org/10.1152/physrev.00040.2004)
The different patterns of staining for phosphorylated Kv4.2 throughout the hippocampus are particularly interesting in the context of the functional consequences of Kv4.2 phosphorylation. First, these data suggest that phosphorylation might serve as a mechanism for targeting. For example, amino-terminal PKA phosphorylation may act as a tag for a discrete pool of Kv4.2 to enter the stratum lacunosum moleculare. Second, as phosphorylation may regulate channel biophysical properties, differential phosphorylation of Kv4.2 in the dendrites of pyramidal neurons may confer unique biophysical properties upon particular dendritic input layers. The potential role that Kv4.x channels play in regulating neuronal synaptic plasticity is addressed in section VI.

VI. CELL BIOLOGY AND REGULATION IN NONNEURONAL SYSTEMS

A. Cardiovascular System

The A-type K⁺ current is a critical regulator of the excitable cells of the heart. Various K⁺ currents determine the amplitude and duration of action potentials in the myocardium. Figure 6 shows a cardiac action potential, which is much longer in duration than a neuronal action potential, and the corresponding K⁺ currents that participate in repolarization of the action potential. The cardiac action potentials are initiated by Na⁺ channels that depolarize the membrane and activate voltage-gated Ca²⁺ and K⁺ channels. The K⁺ currents characterized in the heart are highly variable, depending on the species and the specific region of the heart. This subject is quite complicated and has been discussed with great sophistication previously (140); therefore, we will not cover this issue here. For the purposes of this review, we will concentrate on the Iₒ in the heart, which, similarly to A-type K⁺ current, activates and inactivates rapidly. A great amount of variability across species exists in Iₒ as well as K⁺ channel subunits that contribute to Iₒ, an indication of the different requirements for cardiac function in mammalian systems.

Table 2. Summary of immunoreactivity in the hippocampus proper (CA1 and CA3) and the dentate gyrus (DG).

<table>
<thead>
<tr>
<th>Kinase</th>
<th>CA1</th>
<th>CA3</th>
<th>DG</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>so sp sr</td>
<td>so sp sl</td>
<td>ml gl h</td>
</tr>
<tr>
<td>ERK</td>
<td>+</td>
<td>- + + -</td>
<td>- - -</td>
</tr>
<tr>
<td>Carboxy-terminal PKA</td>
<td>+</td>
<td>- + - +</td>
<td>+ - + +</td>
</tr>
<tr>
<td>Amino-terminal PKA</td>
<td>-</td>
<td>- - + +</td>
<td>- - ± -</td>
</tr>
</tbody>
</table>

+, immunoreactivity is prominent and readily visible in this area; ±, immunoreactivity is moderately detectable in this area; −, immunoreactivity is minimal in this area; so, stratum oriens; sp, stratum pyramidale; sr, stratum radiatum; sl, stratum lacunosum molecular; ml, molecular layer; gl, granule cell layer; h, hilus. [Adapted from Varga et al. (204).]
mals of different sizes. In rodents, $I_{to}$ appears to be the major repolarizing current, whereas in higher mammals $I_{to}$ is responsible only for the rapid repolarization phase (166). $I_{to}$ has now been divided into two distinct transient outward $K^+$ currents, $I_{to,f}$ and $I_{to,s}$, which are differentially distributed in the myocardium. These currents are differentiated based on their rate of inactivation and recovery from inactivation (29, 207, 221). $I_{to,s}$ appears to be formed by Kv4.3 channels (150, 201, 212). $I_{to,f}$ has been characterized in ventricular myocytes as well as atrial cells from various species (140). While there is substantial variability across species, considerable evidence suggests that the Kv4.x family of $K^+$ channel proteins are the primary subunits that underlie $I_{to,f}$ in most species (50).

In rodents, it appears that Kv4.2 and Kv4.3 coassemble with KChIP2 to form $I_{to,f}$ currents of myocytes in the ventricle (69). Both Kv4.2 and Kv4.3 are expressed in mouse ventricle, and antisense oligonucleotides directed against Kv4.2 and Kv4.3 attenuate ventricular $I_{to,f}$ of the mouse (58, 69). One interesting aspect of the rodent ventricle is that the kinetics of the $I_{to,f}$ vary across the ventricular wall. Interestingly, Kv4.2 mRNA expression varies through the ventricular wall of the rat, a gradient that parallels this regional difference in $I_{to,f}$ (51, 69). Moreover, Western blot analysis reveals that the expression of Kv4.2 protein parallels the regional heterogeneity in $I_{to,f}$ density, whereas Kv4.3 and KChIP2 are uniformly expressed in adult mouse ventricle. Finally, KChIP2 knockout mice show a complete loss of ventricular $I_{to,f}$ and an increase in action potential duration, and these animals are highly susceptible to arrhythmias (108). This issue will be revisited in section VIII.

In contrast, Kv4.2 appears to be the only primary subunit that underlies $I_{to,f}$ in atrial myocytes of rodents. Thus $I_{to,f}$ is selectively eliminated in atrial myocytes by Kv4.2 antisense oligonucleotides (27), but not anti-Kv4.3 oligonucleotides. In addition, a transgenic mouse expressing a Kv4.2 dominant negative shows no $I_{to,f}$ in atrial myocytes (220, 221). Taken together, these data suggest that Kv4.2 and Kv4.3 heteromultimerize to form the $I_{to,f}$ of the rodent ventricle, but only Kv4.2 participates in $I_{to,f}$ of rodent atrium.

Different $K^+$ channel subunits appear to be responsible for the $I_{to}$ of higher mammalian heart versus rodent heart. For example, Kv4.3 appears to be solely responsible as the pore-forming subunit for $I_{to,f}$ of canine and human heart (23, 51). The functional diversity of $I_{to,f}$ of canine and human heart is attributed to differential expression of interacting proteins. Thus a gradient of KChIP2 gene expression that parallels the gradient in $I_{to,f}$ expression has been seen in both canine and ferret ventricle (148, 161, 162). In contrast, Kv4.3 mRNA was expressed at equal levels across the ventricular wall. A similar gradient of KChIP2 expression was found across the ventricular wall of human heart, but not rat heart. Another study, however, reported a steep gradient of KChIP2 mRNA, but no differential expression of KChIP2 protein (47). Therefore, while it is clear that KChIP2 is a component of $I_{to}$ in canine and human heart, it is unclear whether differential expression of KChIP2 protein underlies the regional differences of $I_{to}$.

Finally, another interacting subunit, NCS-1 protein (see sect. iv), appears to be developmentally regulated in the heart (139). Cardiac NCS-1 protein expression levels are much higher in fetal and neonatal mouse hearts than in the adult. In addition, NCS-1 colocalizes with Kv4.2 in ventricular myocytes at the sarcolemma and is most likely an important regulator of Kv4.x subunits in the heart. The role of the developmental regulation at this point is unknown.

B. Smooth Muscle

In addition to the heart and nervous system, A-type $K^+$ currents have been characterized in a variety of types...
of smooth muscle cells (see Ref. 9 for a review). A-type K\(^+\) currents have been characterized in the vascular smooth muscles of various species, as well as genitourinary and gastrointestinal (GI) smooth muscle cells (9). While the exact function of the current in smooth muscles has yet to be clarified, it most likely plays a role in the regulation of cellular excitability. Kv4.1–3 primary subunits as well as \(\beta\)-subunits have been detected in rat mesenteric, tail, and pulmonary arteries (218, 219). Indeed, a long splice variant (with a 19-amino acid insert compared with brain) of Kv4.3 was first cloned from rat vas deferens and is found in rat aortic smooth muscle as well as other smooth muscles (145). In addition, Kv4.3 transcripts are twice as abundant as Kv4.1 and Kv4.2 in rat uterus. Interestingly, this expression is plastic, and Kv4.3 transcripts are dramatically reduced in response to estrogen exposure during pregnancy (187). Transcripts for all Kv4.x channels have been localized to the rodent GI tract, including the antrum, jejunum, and colon (7, 8, 10).

Relatively few studies have been performed on Kv4.x interacting proteins in smooth muscle. It is known that KChIP1 and KChIP3 are expressed in mouse GI smooth muscle (144). Similar to the gradient seen in the heart, a gradient in KChIP expression mediates a gradient in A-type K\(^+\) current density in mouse colonic and jejunal myocytes (8, 144).

C. Lung

A recent study has also identified Kv4.x channels in alveolar epithelial cells in the lung (112). mRNA analysis revealed that all three \(\alpha\)-subunits messages are present in these cells. Thus far, however, only Kv4.2 and Kv4.3 proteins have been identified in these cells. Both Kv4.2 and Kv4.3 are localized to the apical membrane of alveolar epithelial cells, which are involved in gas exchange in the epithelium. Interestingly, Lee et al. (112) also found mRNA for all three \(\beta\)-subunits (Kv\(\beta\)1.1, 2.1, and 3.1) as well as two KChIPs (KChIP2 and KChIP3) expressed in lung epithelium. Although the function of Kv4.x channels in alveolar epithelial cells is unknown, it has been speculated that they are involved in K\(^+\) secretion into the alveolar space, or that they may act as an oxygen sensor (143).

D. Other

Finally, the mRNAs for Kv4.1 and Kv4.3 have been found in a variety of other tissue including skeletal muscle, spleen, thymus, bone marrow, liver, pancreas, prostate, and kidney tissue (86). However, we are not aware of any studies that have revealed Kv4.x protein in these regions.

VII. CELL BIOLOGY AND REGULATION IN NEURONS

A. Distribution of Kv4.x Subunits in the Brain

We focus in this section on the role of Kv4.x channels in the hippocampus because it has been most widely studied, but, of course Kv4.x subunits are expressed in numerous neuronal types throughout the mammalian central nervous system (CNS). We will briefly summarize Kv4.x channel mRNA expression patterns in the brain. Pioneering studies by Serodio and Rudy (177) investigated the distribution of the mRNA transcripts encoding Kv4.1, Kv4.2, and Kv4.3 subunits in the adult rat brain using in situ hybridization and histochemistry methods. In general, Kv4.1 expression in the CNS appears to be quite low compared with Kv4.2 and Kv4.3 expression. The expression patterns of Kv4.2 and Kv4.3 messages are complicated, manifesting selective subtype expression patterns in some cells and overlapping expression in others. Serodio and Rudy (177) found that Kv4.2 transcripts are the predominant form in cells in the caudate-putamen, pontine nucleus, and several nuclei in the medulla. In contrast, neurons in the substantia nigra pars compacta, the restrosplenial cortex, the superior colliculus, the raphe, and the amygdala express mainly Kv4.3. In the olfactory bulb Kv4.2 dominates in granule cells and Kv4.3 in periglomerular cells. Kv4.2 is the most abundant isoform in hippocampal CA1 pyramidal cells, although there is some expression of Kv4.3 in a subset of CA1 pyramidal neurons. Only Kv4.3 appears to be expressed in CA1 interneurons. Both Kv4.2 and Kv4.3 are abundant in CA2-CA3 pyramidal cells and in granule cells of the dentate gyrus, which also express Kv4.1. In the dorsal thalamus Serodio and Rudy (177) observed strong Kv4.3 signals in several lateral nuclei and found that medial nuclei express Kv4.2 and Kv4.3 at moderate to low levels. In the cerebellum Kv4.3, but not Kv4.2, is expressed in Purkinje cells and molecular layer interneurons. In cerebellar granule cells, both Kv4.2 and Kv4.3 are expressed. Finally, it is worth noting that Kv4.x channels appear to have a neuron-specific expression in the CNS, and their subcellular distribution is limited to the somato-dendritic compartment in almost all cases examined.

While the expression patterns of Kv4.2 and Kv4.3 transcripts and protein reveal expression throughout the brain, few areas have been studied functionally. We will briefly discuss what is known about the correlation of A-type K\(^+\) currents and Kv4.x channel expression in the brain. The pyramidal cells of the hippocampus have been studied in great detail and will be specifically discussed in section VII/2.
current in this region (184). An A-type K\(^+\) current also exists in inhibitory interneurons of the stratum oriens of area CA1 of the hippocampus. RT-PCR reveals that Kv4.3 is the likely candidate for the pore-forming subunit of this current (117, 124). Functional studies have also revealed an A-type K\(^+\) current in dopaminergic neurons of the substantia nigra that contributes to pacemaker control of their tonic activity. Interestingly, the A-type K\(^+\) current density is tightly correlated with the frequency of tonic spontaneous activity of the dopaminergic neurons (118). Moreover, RT-PCR confirmed that this current was composed of Kv4.3 primary subunits, and Kv4.3 abundance is coupled to A-type K\(^+\) current density (118).

A similar correlation of A-type K\(^+\) current density and Kv4.2 expression has been shown in neurons of the basal ganglia and forebrain, suggesting that Kv4.2 is the major constituent of the A-type K\(^+\) current in these neurons (197). Interestingly, significant differences were seen in the voltage dependence and kinetics of the A-type K\(^+\) currents in these neurons, even though Kv4.2 appears to be the major pore-forming subunit. This suggests that other mechanisms, including auxiliary proteins or post-translational modification, may contribute to A-type K\(^+\) current variability in these neurons.

Confocal and electron microscopy studies demonstrate that Kv4.2 is highly expressed in the plasma membrane of the cell bodies and dendritic processes of magnocellular neurons in the supraoptic nucleus of the hypothalamus (6). This study also suggests that Kv4.2 in supraoptic neurons is concentrated in postsynaptic plasma membranes found adjacent to presynaptic terminals. However, further research is needed to confirm whether these concentrations of Kv4.2 coincide with synapses. Electrophysiological studies have revealed that these neurons express an A-type K\(^+\) current that contributes to the excitability of these neurons (59, 170). Finally, recent work has shown that an A-type K\(^+\) current in the mitral cells of the olfactory bulb regulate backpropagating action potentials similar to pyramidal cells of the hippocampus (discussed below). This modulation has implications for the inhibitory circuits in the olfactory bulb (41). Together, these studies suggest that, indeed, Kv4.2 and Kv4.3 underlie A-type K\(^+\) currents throughout the nervous system and these currents participate in regulation of the excitability of a variety of neurons.

**B. K\(^+\) Channels in Neuronal Information Processing**

In this section we address the role of Kv4.x channels in the context of neuronal function. We consider the role of A-type K\(^+\) currents in general and Kv4.x channels in particular in regulating neuronal membrane excitability. We discuss the function these processes play in neuronal information processing. We highlight recent discoveries that A-type K\(^+\) current regulation may play a role in regulating the induction of NMDA receptor-dependent synaptic plasticity in the hippocampus. We discuss this topic because this area has received considerable attention due to its likely importance in hippocampus-dependent cognitive processing in the intact animal. Furthermore, neurotransmitter regulation of Kv4-family channels in hippocampal pyramidal neuron dendrites exemplifies an area where breakthroughs are being made in understanding the molecular and cellular basis of the plasticity of synaptic function in the CNS.

To fully understand the role of A-type K\(^+\) currents in synaptic plasticity, we briefly discuss the mechanisms of synaptic plasticity. The predominant excitatory neurotransmitter in the mammalian CNS is glutamate. Glutamatergic synaptic transmission underlies both baseline cell-cell communication between neurons and the capacity for alterations of synaptic strength for which CNS neurons are so notable. By and large, baseline synaptic communication is mediated by one family of glutamate receptors (the AMPA/kainate family) and synaptic plasticity is mediated by the NMDA subtype of glutamate receptor.

The NMDA receptor is gated by the ligand (glutamate) as well as voltage, through a voltage-dependent block by Mg\(^{2+}\). NMDA receptor activation allows Ca\(^{2+}\) influx into the cell. The increase in intracellular Ca\(^{2+}\) due to NMDA receptor activation leads to a long-lasting enhancement of synaptic strength at many synapses in the CNS, a phenomenon known as long-term potentiation (LTP). LTP is at present the strongest candidate mechanism for a cellular process contributing to memory formation in the CNS.

The NMDA subtype of glutamate receptor is the prototype “cognitive molecule.” This receptor has immediate appeal in the context of molecular information processing because it can serve as a coincidence detector. Thus the NMDA receptor is selectively opened upon two simultaneous actions: binding of glutamate and depolarization of the membrane in which it resides. It is this second requirement that is relevant in the present context. One theme that is beginning to emerge from work on Kv4.x channels is that these channels can provide the neuron with additional information processing capacity; they do this through controlling the depolarization that the NMDA receptor senses.

It is widely known that action potentials triggered near the cell body of neurons propagate along axons, allowing intercellular communication with downstream targets. Less generally appreciated is the fact that in many CNS neurons action potentials also propagate through neuronal dendrites as well. These dendritic “back-propagating” action potentials are increasingly becoming recognized as an important means of electrical communication within neurons, signaling the proximal and distal...
dendritic tree that the cell has been sufficiently stimulated to fire an action potential. In dendrites of hippocampal pyramidal neurons, a principal model for studying the role of back-propagating action potentials (b-APs), b-APs become progressively smaller in amplitude the further they invade the distal dendrites (121, 134, 189, 228). This decrement is primarily attributable to an increasing density of A-type $K^+$ currents in dendrites (78, see Fig. 7). Thus anything regulating the voltage-dependent properties and/or density of A-type $K^+$ currents can have a direct effect on b-AP amplitude in pyramidal neuron dendrites. We will return to the computational power conferred by these regulatory processes later in this section.

A-type $K^+$ currents in dendrites regulate local membrane depolarization at dendritic spines as well as modulate the arrival and/or effects of dendritic b-APs. This aspect of $K^+$ current function is most easily visualized by considering what are called “silent” synapses, synapses that have NMDA receptors but no AMPA receptors. Activation of AMPA receptors by binding of glutamate results in a membrane depolarization called an excitatory postsynaptic potential (EPSP). Because NMDA receptors are voltage dependent, they cannot be activated if the membrane is not sufficiently depolarized. This requirement is usually provided by AMPA receptor. However, as silent synapses lack AMPA receptor, NMDA receptors are not activated unless depolarization is provided by another mechanism. Any depolarization that the NMDA receptor senses in a silent synapse must of necessity come from a distal origin. Thus $K^+$ currents may have a direct and pronounced effect on synaptic plasticity by controlling the membrane potential detected by NMDA receptors (see Fig. 8).

In this vein, recent models view A-type $K^+$ currents as partners of the NMDA receptor. This is particularly important in our view because $K^+$ currents, in particular A-type $K^+$ currents such as those encoded by Kv4.x family members, are themselves subject to modulation by protein kinases and interacting proteins as described in sections IV and V of this review. Through this modulation they can pass along this biochemical information to the NMDA receptor in the form of an altered membrane depolarization.

**C. Neuromodulatory Effects on A-Type $K^+$ Currents in Hippocampal Pyramidal Neurons**

In this section we describe one example of how these mechanisms operate in the context of the functioning neuron. We will illustrate our discussion by focusing on neurotransmitter modulation of A-type $K^+$ currents in the dendritic membrane of hippocampal pyramidal neurons. We have chosen to highlight this system for several rea-
sons. First, this is the model system that has been studied most extensively at the biochemical level. Second, Kv4.x channels in neurons generally have a somato-dendritic distribution, and sophisticated dendritic attached-patch recording procedures have allowed dendritic A-type K$_{H11001}$ currents to be studied in great detail in hippocampal pyramidal neurons. Finally, we find this system of interest because the cellular physiology may be applicable to the physiology of the whole animal, for example, behavioral output in the context of learning and memory. We will specifically focus on phosphorylation-mediated processes because phosphorylation is a recurring motif for regulation of the properties of K$_{H11001}$ channels. Therefore, phosphorylation of the primary or interacting subunits is the most likely mechanism by which neurotransmitter receptor-coupled and activity-dependent second messenger systems impact A-type K$^+$ current function. Thus studies of Kv4.x regulation may serve as a model for K$_{H11001}$ channel regulation in a variety of subfamilies and cellular systems.

In the intact animal the hippocampus receives numerous input fibers that provide modulation using the neurotransmitters norepinephrine (NE), dopamine (DA), serotonin (5-HT), and ACh. These neurotransmitters increase the likelihood of LTP occurrence at CA1 pyramidal neuron synapses (also known as Schaffer collateral synapses) as well as enhance the magnitude of LTP that is induced. In one example studied extensively by Tom O’Dell’s laboratory (209), 5-Hz stimulation of Schaffer collateral synapses, for 3 min, gives essentially no synaptic potentiation. Coapplication of isoproterenol, a $\beta$-adrenergic receptor agonist that mimics endogenous NE with 5-Hz stimulations, on the other hand, can induce potentiation (209, 215). The magnitude of LTP induced by physiological stimulation protocols that normally evoke modest LTP is also augmented by $\beta$-adrenergic agonists. Similar effects can be observed with activation of various ACh and DA receptor subtypes as well.

The basis for this modulation is complex, but one known site of action of neuromodulators is regulating b-APs in pyramidal neuron dendrites. All of the neurotransmitters described above that modulate LTP induction can modulate the magnitude of b-APs, specifically these agents increase the magnitude of the depolarization delivered by the b-AP (76–78). Augmentation of b-APs is a means by which these neurotransmitters can enhance membrane depolarization and thereby enhance NMDA receptor opening.

How does this augmentation of the b-AP occur? We and our collaborators have proposed a model in which modulation of A-type K$^+$ currents in distal dendrites of the hippocampus critically regulates the magnitude of LTP that is induced. In one example studied extensively by Tom O’Dell’s laboratory (209), 5-Hz stimulation of Schaffer collateral synapses, for 3 min, gives essentially no synaptic potentiation. Coapplication of isoproterenol, a $\beta$-adrenergic receptor agonist that mimics endogenous NE with 5-Hz stimulations, on the other hand, can induce potentiation (209, 215). The magnitude of LTP induced by
shifted in the depolarizing direction, leading to a decrease in channel open probability. This decrease in A-type K⁺ current amplitude in response to any given voltage step increases dendritic excitability and b-APs in dendrites. Recent work has shown that the alterations in A-type K⁺ current voltage dependence caused by application of PKA, PKC, or β-adrenergic receptor activators is secondary to activation of ERK/MAPK (210, 228). In addition, it is worth noting that regulation of A-type K⁺ currents in dopaminergic midbrain neurons caused by glial-derived neurotrophic factor (GDNF) is mediated by ERK/MAPK (225). Overall, these observations indicate that A-type K⁺ current modulation of dendritic membrane potential is regulated by cell surface neurotransmitter receptors coupled to ERK activation. The implication of this, as will be discussed in more detail below, is that neuromodulation of A-type K⁺ currents could serve a critical role in controlling b-APs and local membrane electrical properties. This mechanism allows indirect but critical control over the membrane depolarization necessary for NMDA receptor activation.

What is the molecular basis for this regulation of A-type K⁺ currents? As described in section I, Kv4.x subunits are the key components underlying the rapidly inactivating K⁺ currents operating at subthreshold membrane potentials in neurons, the neuronal A-type K⁺ currents. In addition, Kv1.4, Kv3.4, and other members in the Kv1 family may also contribute to formation of A-type K⁺ currents, but several lines of evidence have suggested that Kv4.x subunits are the best candidate for primary subunits that contribute to dendritic A-type K⁺ currents of hippocampal CA1 pyramidal neurons. In brief, this evidence is as follows: the similarity of biophysical properties between the currents mediated by heterologously expressed Kv4.x channels and native A-type K⁺ currents in dendrites (11, 91, 134, 178, 188), the subcellular distribution of Kv4.2 revealed by immunostaining (122, 181), and the similarity in regulation by certain protein kinases between Kv4.x subunits and dendritic A-type K⁺ currents (3, 12, 228). Although the above evidence suggests a correspondence between Kv4.2 subunits and the dendritic A-type K⁺ currents, the most conclusive evidence will eventually come through the use of genetic manipulation. At present, several labs are working on introducing dominant negative mutations of Kv4.2 into hippocampal pyramidal neurons (L. L. Yuan, personal communication). In a similar vein a Kv4.2 knock-out mouse line has been created, and efforts are being made to determine if the dendritic A-type K⁺ current of CA1 pyramidal neurons is diminished in these mutant animals (Yuan, personal communication). We await future results from these experiments to determine the molecular identity of the channels encoding CNS neuronal A-type K⁺ currents. However, the current evidence indicates that Kv4.2 subunits are localized to dendrites in CA1 pyramidal neurons and are likely the pore-forming subunit of dendritic A-type K⁺ currents in this region.

Moreover, Kv4.2 is a substrate for ERK in vitro and in hippocampal pyramidal neurons (3); thus Kv4.2 is a target for regulation by ERK/MAPK. This evidence provides a specific mechanism for neurotransmitter modulation of dendritic A-type K⁺ currents. Consistent with this hypothesis, in recent studies in collaboration with Dan Johnston’s laboratory, we found that activation of PKA and PKC, as well as stimulation of β-adrenergic receptors, leads to ERK activation and phosphorylation of Kv4.2 by ERK in hippocampal area CA1 (228). As stated above, modulation of A-type K⁺ currents by PKA, PKC, and β-adrenergic receptors is secondary to ERK activation. This mechanism is a basis for controlling b-APs in pyramidal neuron dendrites.

Our working hypothesis is that kinase phosphorylation of Kv4.2 decreases the probability of channel opening or the number of channels in the dendritic membrane. Once these channels in a particular region of a dendrite are rendered nonfunctional due to phosphorylation, the ability of a b-AP to invade that particular dendrite increases. This allows, or increases the likelihood of, NMDA receptor activation and Ca²⁺ influx locally, and thus controls the induction of LTP at that synapse.

This mechanism is likely particularly important in the “theta-type” LTP described above, i.e., 5-Hz stimulation induced LTP (209, 215). This is particularly interesting because theta-frequency stimulation mimics an endogenous CA1 neuron firing pattern that rodents manifest when they are learning about their environment. Theta-frequency stimulation causes action potential bursting in area CA1 cells, which can back-propagate into the dendrites and depolarize synapses. Several groups, including the laboratories of Eric Kandel, Danny Winder, and Tom O’Dell, have shown that blocking ERK activation in mouse area CA1 blocks not only the complex spike bursting seen with the theta-frequency stimulation protocol, but also the LTP that is so induced (209, 215). Moreover, Danny Winder’s group (215) has found that β-adrenergic receptor-mediated modulation of LTP induced with theta-frequency stimulation is blocked by inhibitors of ERK activation. It seems likely that blocking ERK in these experiments decreases the phosphorylation of Kv4.2, leading to an increase in the open probability of the channel. These findings are consistent with a model wherein ERK regulation of membrane electrical properties, via phosphorylation of Kv4.2 channels, regulates b-APs and controls NMDA receptor activation.

What is the physiological role of this complex β-adrenergic receptor regulation of Kv4.2? We believe that it contributes to increasing the sophistication of information processing that can be achieved by the hippocampal pyramidal neuron. Specifically, it can allow for three-way coincidence detection. Consider the following example.
This example is a combination of the NMDA receptor in its classical role as a detector of coincident membrane depolarization plus synaptic glutamate, plus β-adrenergic receptor activation of ERK, and K+ channel regulation by ERK. The model draws directly from data published by Danny Winder and his collaborators (215), Tom O’Dell’s group (209), and us and several of our colleagues (3, 78, 132, 210, 228). The model is schematized in Figure 9.

Imagine that LTP will be triggered by a b-AP, caused in response to a strong, action potential-triggering input at a distal synapse, coupled with local synaptic glutamate. As we have discussed, LTP results because the NMDA receptor senses b-AP-associated membrane depolarization coupled with synaptic glutamate at the synapse of interest. In addition, imagine that A-type K+ currents would limit the capacity of the b-AP to reach the synapse and thus depolarize the NMDA receptor (see Fig. 9), except that a β-adrenergic receptor has activated ERK and downregulated these channels. Thus the β-adrenergic receptor has gated the b-AP and allowed it to enter the relevant dendritic region. This allows NMDA receptor activation, and the resulting calcium influx through the NMDA receptor is sufficient to cause robust synaptic LTP.

In this example a strong synaptic input at a distal synapse, plus a weak synaptic input, plus one neuromodulatory input (NE), has uniquely triggered lasting synaptic plasticity: three-way coincidence detection. It is a molecular analog of a common behavioral situation: an aroused animal (NE) receiving a salient environmental cue at the peak of the theta-rhythm (strong synaptic input) coupled with a second sensory signal (weak synaptic input). The resulting increase in synaptic strength might contribute to the animal forming an associative memory for the event.

The point of this example is to illustrate how the molecular complexity of K+ channel regulation can specify that a precise and multifactorial set of conditions be met to trigger synaptic plasticity. This allows for intricate information processing at the synaptic level, and allows for complex decision making at the molecular and cellular levels. The complex biochemical machinery of the synapse allows for a complicated logic to operate in determining whether a persisting effect is triggered in the CNS. The inclusion of A-type K+ current modulation of NMDA receptor function supports a greater sophistication in cellular information processing than could be achieved by the NMDA receptor alone.

Finally, we note that the molecular complexity of LTP induction has implications for memory formation in general terms. The synapse is a complex signal integration machine. It responds to multiple signals, and its recent history, to integrate information and decide whether to change its state. Many molecular factors, including A-type K+ current regulatory mechanisms, come into play; factors that are critical in allowing the synapse sufficient computational power to perform sophisticated information processing.

D. Additional Levels of Complexity: Kv4.x Channels as Multimodal Signal Integrators

Our final comment is that the mechanisms regulating K+ channel function are not limited to allowing three-way coincidence detection; even greater sophistication is theoretically possible. As described above, the effect of NE on hippocampal neuron excitability is mediated by β-adrenergic receptors via activation of the cAMP cascade, ultimately resulting in the activation of PKA and ERK. DA is likely to achieve a similar effect acting through D1 receptors coupled to adenylyl cyclase. However, in hippocampal pyramidal neurons various NE, DA, and muscarinic ACh receptor subtypes can also couple to phospholipase C, leading to activation of PKC. PKC activation also leads to activation of ERK in the hippocampus. Overall, then, while the effect of neurotransmitters acting through the PKA/ERK cascade is the best-characterized signal transduction cascade in the hippocampus at present, there is a wide variety of potential effectors that could be utilized by neurotransmitters to achieve hippocampal neuromodulation.
In the case of Kv4.2, this adds an additional layer of complexity because parallel PKC and PKA pathways may directly act on Kv4.2 in addition to their more distal effects through stimulating the ERK cascade (see Fig. 10). This potentially allows for PKA- or PKC-coupled pathways to impinge upon ERK phosphorylation of Kv4.2, an example of signal integration at the level of the ERK cascade and the Kv4.2 α-subunit. By serving as a biochemical signal integrator in this way, additional sophistication is available through which cell surface receptors can regulate the depolarization signal that the NMDA receptor senses. This could allow for even greater selectivity in coincidence detection, through the coordinated regulation of Kv4.2 by multiple neurotransmitter systems.

VIII. PATHOPHYSIOLOGY

A. Epilepsy

Intrinsic membrane properties, in addition to synaptic mechanisms, are known to play a role in the pathologically increased electrical activity associated with epilepsy. As has been discussed above, K⁺ channels contribute significantly to the regulation of membrane excitability (75); thus abnormalities in K⁺ channels are a candidate mechanism in epilepsy. Recent work identifying genes underlying idiopathic forms of epilepsy in humans has revealed some rare cases of mutations of K⁺ channels (38, 114, 129, 185, 186). Mutations in some K⁺ channel-associated proteins have also been linked to an epilepsy phenotype. For example, mice lacking Kvβ2 subunits have spontaneous seizures (126). In humans the Kvβ2 subunit maps to chromosome 1p36. Patients with deletions within this region have complex syndromes, which include severe epilepsy (72). No seizure phenotype has yet been described specifically for mutations in Kv4.x channels; however, since Kvβ2 subunits associate with Kv4.x channels, loss of Kvβ2 could potentially affect A-type K⁺ current and thereby contribute to altered excitability and seizures.

Further evidence for a role of K⁺ channels in epilepsy comes from studies using K⁺ channel blockers which lead to convulsive activity in hippocampal slices or whole animals (4, 43, 61, 172). The K⁺ channel blocker 4-aminopyridine (4-AP) induces epileptiform activity in vitro and seizures in vivo (14, 63). More selective pharmacological inhibition of A-type K⁺ currents using several types of venom from scorpion or snake, as described in section 1, also induces convulsive activity (14, 97, 167).

Alterations in A-type K⁺ currents are associated with some animal models of epilepsy and in human temporal lobe epilepsy. For example, a downregulation of A-type K⁺ current has been described in hippocampal dentate granule cells of several patients with lesion-associated temporal lobe epilepsy (22). The critical role that the A-type K⁺ current plays in regulating the excitability of neurons in the hippocampus suggests that alterations in these currents may contribute to seizures in these patients. Similarly, the high seizure susceptibility in endopiriform nucleus (EN) of piriform cortex compared with more superficial layers (layer II) may be related to layer-specific differences in A-type K⁺ currents. In EN neurons, A-type K⁺ currents have smaller peak amplitudes, and steady-state inactivation is shifted toward more hyperpolarized potentials (18). Finally, the locus of aberrant excitability in a rat model of absence (spike and wave) epilepsy is thought to involve the thalamus (128). Normal oscillatory behavior of thalamic neurons involves a balance between an A-type K⁺ current and a Ca²⁺-mediated inward current (Iₜ). In this epilepsy model, the

![FIG. 10. Signal integration by the ERK/Kv4.2 pathway. See text for discussion.](http://physrev.physiology.org.org)
balance of the A-type K⁺ current and Iₚ is disrupted, which may contribute to spike-and-wave discharges.

Interestingly, alterations in Kv4.2 expression are seen in several animal models of epilepsy. Kv4.2 and Kv1.2 mRNA levels were transiently reduced in the granule cell layer of the dentate gyrus following pentyleneetetrazole-induced generalized seizures (200). In the kainate epilepsy model, Kv4.2 mRNA expression levels also decreased in the dentate granule cell layer 3 h after seizures (60). Expression levels of Kv4.2 mRNA rebounded to levels greater than controls in the dentate 24 h after seizures, while levels were significantly decreased in CA3 at this same time point. Kv4.2 mRNA expression levels did not change in animals that did not exhibit seizures following kainate injection. Additional evidence suggesting a role for Kv4.2 in epilepsy is evident in a model of cortical dysplasia and epilepsy induced by prenatal exposure to methyloxazomethanol (MAM). In this model heterotopic pyramidal neurons in the cortex demonstrate a down-regulation of Kv4.2 channel proteins (36). Consistent with this molecular finding, the heterotopic neurons are hyper-excitible. Although the significance of Kv4.2 alterations in these epilepsy models has not been definitively characterized, it is possible that the increased excitability of these neurons and the decreased seizure threshold seen in these models are due to alterations in expression of Kv4.2 subunits and subsequent downregulation of A-type K⁺ currents.

Alterations in the expression of the KChIP3 auxiliary subunit have been shown in epilepsy. There is a decrease of KChIP3 protein in hippocampal tissue from humans with chronic epilepsy compared with control tissue (81, 125). Furthermore, two studies in animal models of epilepsy show alterations in KChIP3 levels: one study shows a decrease in KChIP3 mRNA 7–8 h after seizure induction (125), while the other shows an increase in KChIP3 protein 24 h after seizure induction (81, 125). Although these two studies show varied results, they suggest alterations in the expression of molecules that compose the Kv4.x supramolecular complex and regulate Kv4.x channel function as a candidate mechanism in epilepsy. In addition to alterations of Kv4.x channel properties, KChIP3 may also function as transcriptional repressor (DREAM), which is another mechanism by which this molecule could contribute to epilepsy. The demonstration of K⁺ channel alterations associated with epilepsy phenotypes suggests that K⁺ channel regulation and expression may be an important determinant in the development of epilepsy; however, the specific role of K⁺ channels in the epilepsy phenotype remains largely undefined. Given that epilepsy is an extremely common neurological disorder and ion channel mutations are documented in only very few of the affected individuals, it is likely that there are other factors that play a role in epileptogenesis. Although there are many possibilities, one interesting consideration is that abnormalities in K⁺ channel expression or regulation at a posttranslational level could contribute to the generation of seizures and potentially epilepsy.

As described in sections v and vii, phosphorylation is an established mechanism for the regulation of ion channels in general and Kv4.x in particular. Therefore, these mechanisms may also contribute to seizure generation through rapid alterations in the function of Kv4.x channels. A number of protein kinase pathways are activated during acute seizure activity including ERK, CaMKII, PKC, and PKA (32). Alterations in these protein kinases during the period of epileptogenesis could lead to rapid changes in channel activity by changing the phosphorylation state of the channel. We should also note that phosphorylation of auxiliary proteins is an additional consideration for rapid alterations in the function and expression of Kv4.x channels in epilepsy.

Recent studies suggest that several pharmacological treatments for epilepsy target K⁺ currents, although none is specifically directed at Kv4.x (34, 206, 233, 234). Given all these considerations, drug development has recently been directed toward modulation of K⁺ channel activity as a mechanism of therapeutic benefit in epilepsy. In our opinion Kv4.x channels and associated proteins should be considered as specific candidate targets for the development of antiepileptic therapies.

B. Alzheimer’s Disease

Changes in Kv4.x channels may also occur in Alzheimer’s disease. Mutations in presenilins have been linked to early-onset, autosomal dominant familial Alzheimer’s disease. Presenilin 1 (PS1) and presenilin 2 (PS2) have been shown to interact with KChIP3 (calsenilin) (39) and therefore the Kv4.x channels; however, the functional consequences of the interaction of presenilin with the Kv4.x complex is currently unknown. Transfection of HEK293 cells or rat ventricular myocytes with PS1 increases the endogenous outward K⁺ currents in these cells. No effect on K⁺ currents was observed when these cells were transfected with a mutant form of PS1 (123), suggesting that mutant PS1 expression would decrease K⁺ currents in neurons relative to normal PS1 expression. Moreover, KChIP3 interacts with both PS1 and PS2, but preferentially interacts with the carboxy-terminal fragment of PS2, the portion of presenilin that is implicated in Alzheimer’s disease (30). At this point, a connection to Alzheimer’s disease is purely speculative, and more research is necessary to determine whether Kv4.x channels play a role in Alzheimer’s disease. Various nonpathological functions of presenilins have been described as well, which include membrane trafficking, amyloid precursor protein (APP) processing, Notch signaling, neuronal plas-
ticity, cell adhesion, and ER calcium homeostasis. The interaction of PS1 and PS2 with KChIP3 suggests that Kv4.x channels may be involved in these processes as well.

C. Cardiac Pathology

As discussed in section VI, the currents formed by Kv4.x channels participate in action potential repolarization in the excitable cells of the heart. These action potentials are responsible for normal myocardial contractility; therefore, fidelity in their firing is extremely important to normal cardiac function. As \( I_{\text{to}} \) plays a role in repolarization of the action potential, a decrease in \( K^+ \) currents that underlie \( I_{\text{to}} \) may prolong the action potential, a common defect found in many cardiac disorders, including hypertrophy, myocardial infarction, heart failure, and arrhythmias. These disorders are complex, and \( I_{\text{to}} \) is not the only current that is altered in these myocardial pathologies. This subject has been discussed elsewhere (141); therefore, we will only discuss the role of \( I_{\text{to}} \) and Kv4.x channels in cardiac pathology and animal models of various cardiac disorders.

Transgenic mice have been used to study the role of \( I_{\text{to}} \) in regulating cardiac action potentials and cardiac pathology. In a transgenic mouse expressing a dominant negative form of Kv4.2 in which a point mutation has been introduced into the pore region, \( I_{\text{to}} \) was eliminated. The ventricular myocytes of these mice display a prolonged action potential. However, these animals appeared normal and did not exhibit arrhythmias (20). Another study eliminated \( I_{\text{to}} \) using transgenic mice expressing a dominant-negative amino-terminal fragment of Kv4.2. In addition to dramatic reductions of \( I_{\text{to}} \), the myocytes of these animals also showed reductions in other \( K^+ \) currents, prolonged action potentials, and increased cell capacitance, an electrical measure of cell size which indicates hypertrophy. These mice developed clinical and hemodynamic evidence of congestive heart failure, such as myocardial cellular and molecular remodeling, hypertrophy, chamber dilatation, and interstitial fibrosis (214).

Cardiac hypertrophy is a general term describing an increase in cardiac mass and myocyte size in response to applied stress. Most studies of cardiac hypertrophy demonstrate a lengthening of the cardiac action potential as well as a decrease in \( I_{\text{to}} \) (71, 102, 130, 155) and a corresponding decrease in Kv4.2 and Kv4.3 mRNA (102, 193). However, some studies have shown that hypertrophy results in increased L-type \( Ca^{2+} \) currents, with no decrease in \( I_{\text{to}} \) (25, 208). Furthermore, a calcineurin inhibitor prevented the increase in \( Ca^{2+} \) current and corresponding hypertrophy, suggesting a central role for calcineurin and not \( I_{\text{to}} \) reduction in cardiac hypertrophy (208). More recently, however, Kassiri et al. (103) demonstrated that reduction of \( I_{\text{to}} \) causes hypertrophy via a calcineurin-dependent pathway. Therefore, they suggest that the prolonged action potential due to decreased \( I_{\text{to}} \) leads to a greater influx of \( Ca^{2+} \), which activates calcineurin and transcription of hypertrophic responsive genes.

Interestingly, chronic hypoxia can block the increase in \( I_{\text{to}} \) density that normally occurs from postnatal day 5 to 15 under normoxic condition in rat ventricular myocytes (68, 100, 101). Thus it is possible that any conditions of hypoxia in myocytes, such as caused by myocardial infarct, could cause a decrease in \( I_{\text{to}} \).

Long-term consequences of myocardial infarction (MI) include significant hypertrophy (described above). Left ventricle myocytes show a decrease in \( I_{\text{to}} \) following MI in rodent models (87, 88, 157, 226). This reduction was greater in epicardial than in endocardial myocytes (226). Furthermore, this decrease in \( I_{\text{to}} \) precedes the onset of hypertrophy, supporting the argument that \( I_{\text{to}} \) reduction contributes to hypertrophy and remains reduced up to 16 wk (88, 163). The changes in \( I_{\text{to}} \) correlated with decreased mRNA and protein levels of Kv4.2 and Kv4.3 (87, 88). Interestingly, ventricular tachyarrhythmias were found in 60% of the rats 3 days after MI. Thus changes in \( K^+ \) current expression associated with the hypertrophied myocardium may play a key role in arrhythmia generation following MI.

Cardiomyopathy observed in rodent models of insulin-deficient type I diabetes include a downregulation of both \( I_{\text{to}} \), and \( I_{\text{to},5} \) in ventricular myocytes (222). In one study, this downregulation of current correlated with a decrease in Kv4.2 and Kv4.3 protein and mRNA expression as early as 14 days after induction of type I diabetes in the rat (156). However, another study found that Kv4.2 mRNA and protein, but not Kv4.3 mRNA, was decreased in the rat ventricle of a diabetic rat model (46).

Atrial fibrillation (AF) is the most common arrhythmia encountered clinically. The development of AF leads to electrical remodeling, such as an increase of the atrial action potential duration and refractory period, which tend to sustain AF. The molecular events leading to electrical remodeling are beginning to be elucidated. Interestingly, \( I_{\text{to}} \) from atrial myocytes of patients with chronic atrial fibrillation is significantly reduced compared with patients with normal sinus rhythm (203). Patients with paroxysmal or persistent AF have decreased Kv4.3 protein and mRNA levels (30, 66), as well as reduced \( I_{\text{to}} \) current densities (66, 98). Likewise, dogs with either chronic atrial fibrillation or brief episodes of fibrillation exhibited significantly reduced \( I_{\text{to}} \) current density and altered kinetics in atrial cells (53). A decrease in \( I_{\text{to}} \) was paralleled by a decrease in Kv4.3 mRNA and protein in dogs that were subjected to rapid atrial pacing to mimic tachycardia (229). A similar reduction in \( I_{\text{to}} \) and Kv4.3 mRNA expression was observed in rabbits 24 h after commence-
ment of rapid atrial pacing. Kv4.2, Kv4.3, and KChIP2 mRNA levels were not affected (26). In the rat atrium, 4 h of rapid pacing gradually and progressively decreased mRNA levels of both Kv4.2 and Kv4.3 (223). Thus chronic or nonsustained atrial tachycardia alters atrial ion channel gene expression, thereby altering ionic currents in a fashion that promotes the perpetuation of AF.

Changes in auxiliary subunits that interact with Kv4.x channels also appear to be sufficient to reduce \( I_{\text{to}} \) and lead to cardiac arrhythmias. For example, mice lacking the auxiliary subunit KChIP2 exhibit a complete loss of \( I_{\text{to}} \) and an increase in action potential duration. These animals are also highly susceptible to cardiac arrhythmias (108).

Because decreased \( I_{\text{to}} \) is associated with arrhythmias and myocardial hypertrophy, regulation of \( I_{\text{to}} \) represents a powerful target of pharmacological intervention in the treatment of cardiac pathology. For example, chronic treatment of post-MI rats with the thyroid hormone analog 3,5-diiodothyropropionic acid (DITPA) restored \( I_{\text{to}} \) density and Kv4.2 and Kv1.4 expression to levels observed in sham-operated controls (213). Other membrane currents were unaffected by DITPA treatment. Furthermore, action potential durations were prolonged after MI and restored to normal durations after DITPA treatment. These results demonstrate that DITPA may be useful in treating disorders associated with \( I_{\text{to}} \) downregulation.

As mentioned above, calcineurin contributes to the mechanisms mediating cardiac hypertrophy. Interestingly, the calcineurin inhibitor cyclosporin A can decrease the electrophysiological changes associated with cardiac hypertrophy and post-MI remodeling. Treatment of animals with cyclosporin A partially blocked the decrease in Kv4.2 and Kv4.3 mRNA levels and \( I_{\text{to}} \) density in left ventricle myocytes post-MI as well as ameliorating post-MI hypertrophy (46). Moreover, treatment of the transgenic mice expressing the truncated Kv4.2 subunit with either cyclosporin A or verapamil, which blocks voltage-gated \( \text{Ca}^{2+} \) channels, reduced the cardiac pathology observed in these animals (165).

Finally, in a rodent model of renovascular hypertension, Kv4.2 and Kv4.3 channel mRNA expression is reduced in ventricular myocytes. Chronic administration of captopril, an angiotensin-converting enzyme inhibitor, blocked the development of hypertension and the suppression of Kv4.2 and Kv4.3 mRNA levels. Furthermore, captopril significantly increased Kv4.2 mRNA in sham-operated rats (193). Given these results, drug development directed at modulation of \( I_{\text{to}} \) is a candidate target for treatment of cardiac pathologies. Therefore, drugs that directly regulate Kv4.x channels and their associated proteins should be considered.

**IX. SUMMARY AND FUTURE DIRECTIONS**

There has been an explosion of information concerning the structure and function of K\(^+\) channels in general since the Jans’ laboratories first cloned the Shaker channel. Although much of the high-resolution structure-function data were not obtained from Kv4.x channels specifically, information gleaned from other K\(^+\) channels has clearly benefited research in Kv4.x channels. Hopefully future investigations will include specific studies of the molecular structure and biophysics of Kv4.x channels.

New information of this sort is particularly important for Kv4.x channels because of the extreme complexity of their regulation, as we have discussed in this review. In our view this has been one of the most surprising aspects of the recently emerging data concerning Kv4.x channel function. The sheer number of interacting and modulatory proteins was unexpected, as was the diversity of their modulatory effects on channel trafficking and biophysics. The importance of these proteins for Kv4.x channel regulation is just now being appreciated. Similarly, the number and diversity of cellular signal transduction pathways regulating A-type K\(^+\) current is constantly expanding. The degree to which ancillary subunits and posttranslational modifications interact in channel regulation also appears to be an area of growing importance. In the limit, we believe that a fundamental change in perspective concerning A-type K\(^+\) currents is called for; we need to begin to think of the pore-forming subunits as one small part of a very large and quite complicated supramolecular complex that performs a variety of functions in the cell.

Subcellular trafficking issues are also likely to be a very fruitful area of future investigation for Kv4.x channels. Their unique distribution patterns in neurons and in the cardiovascular system suggested to the first investigators in the field that issues of localization and trafficking were likely to be important. This presupposition has certainly held up over time. Several investigators studying Kv4.x channels are pioneering efforts to understand the distribution of ion channels in selective subcellular domains. This is one area in which studies of Kv4.x channels may fundamentally contribute to our understanding of cell biology.

A recurrent theme that one may have noticed throughout this review is that a tissue-specific and cell-specific distribution of each Kv4.x protein exists. For example, while rodent atrial cells express Kv4.2 exclusively, ventricular cells express both Kv4.2 and Kv4.3. What is the purpose of this distinctive distribution of Kv4.x channels? While the current mediated by the different Kv4.x proteins are very similar, there are subtle differences in their kinetics. For example, in the same expression system Kv4.3 currents inactivate slower and recover from inactivation faster than Kv4.2 currents (69). Furthermore, interactions with auxiliary subunits (dis-
cussed in sect. v) or posttranslational modification (discussed in sect. v) introduce greater variability in channel kinetics. These kinetic differences cause alterations in the A-type current which would translate into differences in cell excitability. This mechanism may allow cells to regulate their excitability based on the physiological demand for that cell.

How do cells regulate levels of one Kv4.x protein versus another? One potential mechanism could be through different rates of production (i.e., one Kv4 protein may be more dynamically produced versus another being more static). This regulation could be at the transcriptional level with cell-specific differences in the control of gene expression. Levels of protein are also regulated through interactions with auxiliary subunits and posttranslational modification which may alter channel stability. Although these are purely speculative ideas, we expect more research to determine the mechanisms responsible for the cellular segregation of Kv4.x proteins and the functional consequences of their distinct expression pattern in the next few years.

Finally, we note the importance of understanding A-type K⁺ current because of its relevance to the human condition. New treatments for epilepsy, cognitive dysfunction, and cardiovascular disease may arise from an increased understanding of the function and regulation of Kv4.x channels and the A-type K⁺ currents that they form. These important considerations in themselves provide compelling motivation for continued, focused investigation into the molecular and cellular biology of this channel family.

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