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Topical Review

Mechanisms of K^+ Channel Regulation

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Introduction

Potassium channels are ideal for examining the structure/function aspects of ion channel regulation, since their subunits are relatively small proteins, and they assemble into simple oligomeric structures. For all of their structural simplicity, however, they are regulated by a wide range of cellular mechanisms and contribute to a host of cellular processes from setting the resting membrane potential to controlling secretion of hormones. The diversity of regulation includes covalent modifications such as phosphorylation, and a host of noncovalent interactions with ions, nucleotides, oxygen, lipids, G proteins, small charged compounds including spermine, as well as structural stresses such as membrane stretch and flow. Finally, expression of K^+ channels is dynamically regulated in a variety of cell types. Recent molecular dissection of a variety of neurological diseases, including Alzheimer's [47] and human episodic ataxia [3] as well as identification of the defects in the mouse mutants *shiverer*, *Trembler* [180] and *weaver* [135, 118] implicates altered regulation and/or function of K^+ channels. A brief overview of the potential mechanisms regulating K^+ channel activities may provide insights into the contribution(s) of K^+ channels to a variety of aspects of cellular physiology.

A Digression — Structural Classes of K^+ Channels

Because current approaches to understanding the regulation of K^+ channel activity are intimately linked with our developing view of the topological arrangement of K^+ channel proteins, a brief discussion of the structural domains of K^+ channels is in order. Multiple classes of K^+ channels have been identified, but three structural motifs plus variations on the themes have emerged for plasma membrane-localized K^+ channels, and are exemplified by the voltage-gated K^+ channel superfamily (denoted K_v), the inward rectifier K^+ channel superfamily (denoted K_{ir}), as well as several newly described hybrids of the K_v and K_{ir} families, and the minimal K^+ channel, minK.

Voltage-gated K^+ channels (Fig. 1*A*) have a common core structural motif (reviewed in 71) which includes six membrane-spanning segments including an S4 segment which contributes to voltage sensing [133, 100, 174, 114], an H5 segment or P loop [108] which forms at least part of the lining of the conduction pore [195, 142] along with parts of the S6 segment [103] and the S4–S5 loop [164]. The N terminus may contain both a blocking particle (which is involved in fast inactivation [61, 198]), as well as a region which directs subunit assembly and restricts cross-family associations [98, 13, 162]. The large C terminal domain may contain multiple phosphorylation sites, as well as binding sites for a variety of modulators of channel function. The functional channel unit consists of a tetramer of α subunits (either a homomultimer [107] or heteromultimer [150, 67, 181, 141]), and may also include one of the recently identified β subunits [147, 120, 109, 38], with a proposed stoichiometry of $\alpha 4\beta 4$. Coexpression of α subunits with mammalian β subunits results in significant alterations in channel gating kinetics (review, [2]), suggesting that one mode of K^+ channel regulation may be variable association with differentially-expressed β subunits. β subunit family members have significant homologies with the aldo-keto reductase superfamily [28], and have retained

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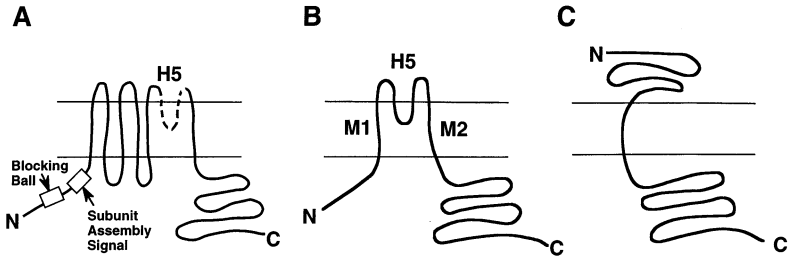

Fig. 1. Schematic representation of the three major structural classes of plasma membrane-localized K^+ channels. (*A*) K_v channels as exemplified by the *Shaker* K^+ channel subfamily; (*B*) K_{ir} channels; and (*C*) minK channels.

the critical residues implicated in cofactor binding, suggesting that they may be pyridine nucleotide-binding proteins with catalytic activity [115]. β subunits may therefore function not only to regulate α subunit inactivation, but in linking K^+ channels to oxidoreductive metabolism. A structurally distinct β subunit has been identified as the charybdotoxin binding site for Ca^{2+}-activated K^+ channels [81, 117]. Regulation of β subunit interactions with α subunits must also be considered when evaluating the effects of a variety of channel modulators, including phosphorylation and binding of regulatory molecules such as ions, nucleotides or G protein subunits.

Inward rectifier K^+ channels (Fig. 1*B*) have two membrane spanning segments, M1 and M2, separated by an H5 equivalent or P loop (reviewed in [33]). A distinguishing characteristic is a large C terminal domain which contributes to the pore [167, 138] and contains sites for binding of regulatory molecules. The N terminal domain may also be involved in binding modulators, as has recently been demonstrated for GIRK-βγ interactions. K_{ir} channels, in analogy with the K_v channels, form tetrameric functional structures, as has recently been demonstrated with tandem constructs [190]. Heteromultimeric assembly may be required for function of some channel subtypes [85, 33, 82, 35]. Functional reconstitution of K_{ATP} channels via coexpression of a new member of the K_{ir} family cloned from pancreatic islets, K_{ir} 6.2 [65] with the sulfonylurea receptor [5, 172] suggests non-K_{ir} proteins may also be required for full reconstitution of in vivo inward rectifier activities.

Recent identification of two ''hybrid'' K^+ channel motifs in yeast and *C. elegans* suggests the potential for additional K^+ channel families related to the K_v and K_{ir} superfamilies. The TOK1 channel identified in yeast via sequence homologies within the P loop is a hybrid containing both a 6 transmembrane K_v-like module linked in tandem with a K_{ir} module, resulting in a total of 8 transmembrane domains and two highly homologous P loops [76]. Interestingly, this channel functions as an outward rectifier, with inward current being blocked by extracellular divalent cations (in a manner analogous to Mg^{2+} block of outward current in K_{ir} channels). A second motif, identified in *C. elegans*, consists of two K_{ir} domains in tandem [154]. Because of the duplication of P loops in both hybrid channels, the functional unit may be a dimer, resulting in a symmetrical, 4-P loop structure, in analogy with the K_v and K_{ir} functional units. It remains to be seen whether these channels are represented in other species, and how their activities are regulated.

minK channels (Fig. 1*C*) represent a third, distinct class of K^+ channels. minK channels display very slow gating kinetics, and have a simple primary structure consisting of a single predicted membrane spanning domain [169, 45]. Although there is some evidence that minK regulates endogenous channels [12] or exists in functional and nonfunctional forms in the membrane (Blumenthal & Kaszmarek, 1994), mutations in the minK amino acid sequence [170] or chemical modification of amino acid side chains [179] directly affect channel activity and gating, implying that the minK protein itself exhibits channel activity. minK channels form functional oligomers in the membrane in response to alterations in membrane potential [179], in a manner reminiscent of pore forming antibiotics such as alamethicin [187, 188, 15].

In addition to the three general families of K^+ channels which have been identified by molecular cloning (Fig. 1), there are plasma membrane-localized channel activities which have not yet been characterized at the molecular level, including O_2, stretch, and Na^+-gated K^+ channels. Further, K^+ channels have been identified within intracellular membranes, and may represent additional gene families. We will here consider the modes of regulation of either cloned channels or endogenous activities, with the goal of identifying common themes and issues which must be resolved before a comprehensive understanding of K^+ channel regulation can be achieved.

Mechanisms of K^+ Channel Regulation

Broadly defined, regulation of K^+ channel activity can encompass both intrinsic regulation (time dependent changes in activity resulting from conformational changes in the channel molecule), and extrinsic regulation (regulation of activity by the binding of a variety of regulatory substances or covalent modification of the channel protein). These two modes of regulation obviously overlap, in that binding of an extrinsic factor (or phosphorylation of the channel) may modify intrinsic regulatory mechanisms.

Intrinsic Regulation of K^+ Channel Activity

The most elegant and obvious example of intrinsic regulation, which may have broader implications as new subunits are discovered, is the binding of the N terminal blocking peptide [61, 198] to a specific ''receptor'' site [68] within the mouth of open *Shaker* K^+ channels. Mutations which remove the blocking ''ball'' eliminate inactivation [61], while application of a soluble peptide ''ball'' restores inactivation [198]. β subunits may also contain a ''blocking particle'' equivalent [147]. Although this type of K^+ channel regulation is inherent within the primary sequence of the protein itself, modification of the efficacy of the blocking particle may result from alterations in either the blocking particle or the ''receptor'' by extrinsic factors such as phosphorylation [31].

Extrinsic Regulation of K^+ Channel Activity

Extrinsic regulation of K^+ channels runs the gamut from protein kinase-mediated phosphorylation to binding of a variety of second messenger and/or regulatory molecules to interaction with G proteins. All of these interactions alter gating and/or permeation through K^+ channels. The following discussion highlights what is known about the various modes of K^+ channel regulation, although there is considerable overlap, and a particular channel type may be mentioned in several sections. For example, Ca^{2+}-activated K^+ channels are most obviously regulated by intracellular Ca^{2+} concentrations, but are also modulated by membrane potential, protein kinase A-mediated phosphorylation, and by direct G protein interactions. In most instances, the hierarchy of regulation in vivo of a particular channel remains to be explored.

Ion-mediated Regulation of K^+ Channel Activity

Many K^+ channels are regulated by divalent cations such as Mg^{2+} and Ca^{2+}. Na^+-dependent K^+ channels have also been functionally characterized. In general, Ca^{2+} and Na^+ facilitate gating of K^+ channels, while Mg^{2+} inhibits a variety of K^+ channels, although Mg^{2+} is obviously required in conjunction with ATP as a substrate for protein kinases, or GTP for activation of heterotrimeric G proteins. Cl^- may also indirectly modulate the activity of some K^+ channels by altering the turnover of G proteins [124]. In this section, we will consider only mechanisms in which the regulatory cation interacts directly with the ion channel.

Ca^{2+}-activated K^+ Channels

The hallmark of Ca^{2+}-activated K^+ (K_{Ca}) channels is the increase in channel open probability, P_o, caused by binding of intracellular Ca^{2+}. K_{Ca} channels can be functionally differentiated according to conductance, i.e., BK or maxi-K channels, intermediate conductance channels, and SK or small conductance channels, as well as by their distinctive pharmacologies and molecular organizations (i.e., associated subunits).

The first K_{Ca} cloned was the *Slowpoke* mutant from *Drosophila* [11, 4]. The cloning of mammalian variants rapidly followed [4, 25]. These channels have a predicted topology similar to K_v channels (Fig. 1*A*). The large C terminal domain of this class of channels is highly variable due to alternate splicing; the various splice combinations yield channels with distinct functional properties when expressed in oocytes [4, 25], and thus may contribute to the diversity of K_{Ca} channel activities observed in vivo. Chimeras between the mouse (mSlo) and *Drosophila* (dSlo) clones have identified two distinct functional domains, a conserved channel core and a C terminal Ca^{2+} binding domain separated by a variable sequence linker [183]. Single channel properties are endowed by the channel core, whereas the affinity and/or Ca^{2+} sensitivity is conferred by the C terminal domain. Maxi-K channels are sensitive to inhibition by peptide toxins such as charybdotoxin and iberiotoxin (review, [19]). This property has been used to identify and clone an associated β subunit [81] which increases both the Ca^{2+} and voltage sensitivities of the α + β complex over that of α alone [117]. The β subunit is not homologous to that which associates with K_v channels, and has a predicted membrane topology consisting of two membrane spanning domains with a large, intervening extracellular domain. The β subunit produces no channel currents when expressed without the α subunit in *Xenopus* oocytes [117]. Maxi-K channels have single channel conductances in the range of 250–300 pS (in symmetrical 140 mM KCl), open probabilities which are enhanced by depolarization, are highly selective, and exhibit high cooperativity for Ca^{2+}-mediated activation (Hill coefficients of 2–4). Ca^{2+} blockade of the conduction pathway upon prolonged depolarizations has been reported [75].

Intermediate conductance K_{Ca} channels have been functionally identified in sensory neurons. They have conductances in the range of 30–60 pS (symmetrical 145 mM KCl), and have a pharmacology which is distinct from maxi-K or SK channels, i.e., insensitivity to TEA, apamin and charybdotoxin [144, 175, 54]. The molecular identity of this class of K_{Ca} channels remains to be established.

SK channels have singe channel conductances of ≤80 pS (in symmetrical 140 mM KCl solutions), may exhibit either voltage-dependent or voltage-independent gating, and in general, have a higher affinity for intracellular Ca^{2+} than maxi-K channels. They are blocked by the bee venom peptide apamin, which has led to identification [159, 160, 95] and cloning [165] of an apamin

binding protein. The 78 kDa apamin binding protein has 4 transmembrane domains (with N and C termini cytoplasmic), an EF-hand Ca^{2+} binding motif in the S2–S3 intracellular loop, and both a consensus PKC phosphorylation site and a leucine zipper within the C terminal domain. Full functional reconstitution of SK channels await identification of the pore-forming α subunit(s).

A strong dependence upon intracellular Ca^{2+} confers upon all types of Ca^{2+}-activated K^+ channels sensitivity to receptor agonists which alter intracellular Ca^{2+} by either mediating release from intracellular stores or inducing influx of extracellular Ca^{2+}. The most significant open questions remain establishing the molecular identities of the various K_{Ca} activities, localization of Ca^{2+} binding sites within the K_{Ca} channel structure(s) and establishing the mechanism(s) of Ca^{2+}-mediated alterations in K_{Ca} channel gating.

Ca^{2+} Modulation of K^+ Channels

A second role for Ca^{2+} in gating of voltage-dependent K^+ channels has been identified. In preparations as diverse as the squid giant axon [8, 7], lymphocytes [50], and *Shaker* A-type K^+ channels expressed in Sf9 cells [9], extracellular Ca^{2+} has been shown to be required for normal functioning, i.e., gating of K^+ channels. Removal of extracellular Ca^{2+} reversibly reduces K^+ selectivity [7, 50], producing a nonselective leak current which can be specifically attributed to the K^+ channel. Armstrong & Miller [9] proposed that the role of Ca^{2+} in K^+ channel closure is to stabilize the association between the 4 α subunits contributing to the pore; in the absence of Ca^{2+} the structure becomes more flexible and thereby less selective. External Zn^{2+} (which may bind to the same external divalent cation site) causes a delay in channel opening, suggesting that it alters the rate(s) of transition(s) among closed states [166]. The Ca^{2+} binding site conferring structural stability as well as normal channel gating has yet to be identified at the molecular level.

Mg^{2+} Modulation of K^+ Channels

A fundamental characteristic of the inward rectifier K^+ (K_{ir}) channel family, i.e., inward rectification, is mediated, in part, by intracellular Mg^{2+}. Intracellular divalent cations, primarily Mg^{2+} [177, 69] but also Ca^{2+} [113], compete with K^+ for access to the pore, preventing or reducing outward current at potentials more positive than the K^+ equilibrium potential [112, 111]. Removal of intracellular Mg^{2+} under either whole cell or single channel recording conditions produces ohmic instantaneous current–voltage relationships for IRK channels [111, 112]. Block by intracellular Mg^{2+} is very voltage-dependent, and results in a flickery, open channel block [111]. Mg^{2+} actually increases the steady-state current through inward rectifier K^+ channels (compared to the absence of Mg^{2+}) by preventing channel closure. Similar mechanisms have been used to explain the contributions of intracellular Mg^{2+} to rectification of ATP-regulated K^+ channels [58, 42], and muscarinic K^+ channels [59].

The recent cloning of several members of the IRK family has permitted structural localization of the site which determines the degree of rectification in the presence of Mg^{2+}. IRK channels can be classified as either weakly rectifying, represented by ROMK1 [57, 125] and K_{ATP} [127], or strongly rectifying, e.g., IRK1 [87] and the muscarinic K^+ channel [189]. Sequence comparisons between weakly and strongly rectifying channels, and chimeras made between ROMK1 and IRK1 suggest that the Mg^{2+} sensitivity can be localized to a site within the M2 transmembrane domain in IRK channels [105, 185]. Weakly rectifying channels contain an aspartic acid residue within M2, while strongly rectifying channels contain an asparagine in the comparable position. An independent mechanism conferring some aspects of rectification and time dependence to the gating of IRK channels was identified concurrently with the Mg^{2+} binding sites, i.e., block by polyamines such as spermidine [102, 41, 40, 189]. Inasmuch as polyamines may be considered organic Mg^{2+} analogues, the mechanisms of rectification induced by polyamines and Mg^{2+} may overlap. This has been confirmed by site-directed mutagenesis in which both Mg^{2+} block and polyamine block are affected in parallel [191].

Extracellular Mg^{2+}, Ca^{2+}, and Sr^{2+} are all capable of causing hyperpolarization-dependent fast, open channel block (reducing unitary conductance) of K_{ir} channels, suggesting that there must also be a divalent cation binding site accessible from the extracellular mileau [19, 163, 93], although the structural determinants of this site have not yet been identified.

Na^+-activated K^+ Channels

Na^+-activated K^+ channels have been functionally identified primarily in brain (snail neurons [134], quail trigeminal neurons [14, 52], crayfish neurons [53], *Drosophila* giant neurons [153], zebra fish hyperstriatal neurons [88], chick brainstem neurons [36], rat olfactory bulb neurons [37], and in various regions of mammalian brain [30, 158]) and heart [74, 106, 149]. These channels are activated by physiological concentrations of intracellular Na^+ (10–180 mM), and are highly selective for K^+. Single channel currents display inward rectification as a result of Na^+ block of outward K^+ currents (in a manner analogous to Mg^{2+} block). Patches containing Na^+-activated K^+ channels exhibit rapid rundown upon excision, suggesting some additional intracellular modu-

latory factors are required for sustained activation [37], although these have not been identified.

Nucleotide-mediated Regulation of K^+ Channel Activity

K^+ channels can be modulated by nucleotides in at least three ways: by direct interaction of the nucleotide with the ion channel, which we will discuss in this section; by phosphorylation; or by indirect mechanisms requiring nucleotides, such as cyclic nucleotide-mediated activation of protein kinases (which also requires ATP) or via GTP-mediated activation of G proteins. Phosphorylation and the indirect mechanisms will be considered in subsequent sections.

ATP-sensitive K^+ Channels

ATP-sensitive K^+ (K_{ATP}) channels have been functionally identified in the membranes of cardiac and skeletal muscle cells, pancreatic β cells, kidney and brain cells [10]. ATP-sensitive K^+ channels are in general highly selective for K^+, require phosphorylation for activation, are inhibited by ATP and stimulated by nucleotide diphosphates. Selective activators and inhibitors of K_{ATP} channels, which include diazoxide and sulfonylureas, respectively, permit pharmacologic modulation of the activity of these channels. The properties of K_{ATP} channels in various cell types are heterogeneous, suggesting a family of related proteins. Indeed, a number of K_{ATP} channels have been cloned, including ROMK1, which is *activated* by ATP and was cloned from kidney outer medulla [57], as well as uK_{ATP}-1 which is ubiquitously expressed, blocked by ATP, and activated by a K_{ATP} channel opener, diazoxide [66]. None of these cloned K_{ATP} channels is sulfonylurea-sensitive, suggesting that additional subunits may be required. Indeed, the K_{ATP} channel from pancreatic islets has been fully reconstituted by coexpression of a newly cloned IRK channel (BIR; $K_{ir}6.2$) plus the sulfonylurea receptor, SUR [65]. The resultant channel has a conductance of 76 pS, is sensitive to block by ATP and sulfonylureas, and is activated by diazoxide. ATP sensitivity is conferred by SUR, which contains ATP/ADP binding sites [17, 5] and is a member of the ATP-binding cassette or traffic ATPase superfamily. Interestingly, expression of either BIR or SUR alone does not result in measurable channel activity.

Functional modulation of K_{ATP} channels by nucleotides is complex. ATP and hydrolysis-resistant ATP analogs inhibit K_{ATP} channel activity in the absence of Mg^{2+}, suggesting ATP hydrolysis is not required. Other nucleotide triphosphates including CTP and GTP inhibit modestly, while nucleotide diphosphates (ADP, UDP, GDP) only inhibit in the absence of Mg^{2+}. In the presence of Mg^{2+}, ADP and GDP actually stimulate the cardiac K_{ATP} channel [94], presumably by decreasing channel sensitivity to ATP-dependent inhibition. Mg-ATP is also required to maintain K_{ATP} channels in an operable state [44], which may be due to the requirement for phosphorylation [129, 46] or may represent a requirement for ATP hydrolysis via SUR in a manner analogous to CFTR [46]. Allosteric interactions between nucleotides and other agents which modulate K_{ATP} channel activity, including extracellular divalent cations such as Cd^{2+} and Zn^{2+} [93], and sulfonylurea drugs [43] have also been observed.

Kinetic models which accommodate all available single channel data require binding of 4 molecules of ATP [126]. Neither opening nor closing rates of the channel in response to ATP concentration jumps is voltage dependent [143], suggesting that ATP allosterically regulates channel gating, rather than blocking the pore directly. The localization of ATP binding sites to the sulfonylurea receptor [5, 17] suggests that, at least for a subclass of K_{ATP} channels incorporating this subunit, ATP sensing may be remote from the pore. In contrast, the ROMK1 channel contains a putative nucleotide binding site similar to that found in cyclic nucleotide-gated channels and CFTR within its C terminal domain [57], and in this case, binding of ATP activates the channel. Further exploration of the subunits constituting the K_{ATP} activities in various cell types will be required before the mechanistic distinctions dictated by the various structural domains can be determined.

Cyclic Nucleotide-activated K^+ Channels

Cyclic nucleotides indirectly modulate a variety of K^+ channels types by virtue of their role in activation of protein kinases. Direct activation of K^+ channels by cGMP has also been described in vascular smooth muscle [146], pancreatic β cells [56], and cortical collecting ducts [182]. The channel has been cloned from the latter [192], and presents a structure which is related to both K_v channels and the cGMP-gated nonselective cation channels [161], containing a *Shaker*like channel core and a C terminal cyclic nucleotide binding domain. Further work is required to determine whether this channel represents the first member of a new subfamily, as well as the details of cGMP-mediated regulation.

Lipid-modulation of K^+ Channels

Lipids (arachidonic acid, arachidonic acid metabolites, fatty acids and phospholipids) affect the activity of a variety of K^+ channels via either direct interactions or via metabolic conversion to signaling molecules such as

prostaglandins and leukotrienes, which then act on their receptors to activate other modulatory pathways. Lipids and other hydrophobic molecules generally also present a third possibility which can be difficult to rule out, namely, alterations in membrane fluidity (i.e., nonspecific perturbations of the system).

Arachidonic acid and fatty acids activate outwardly rectifying K^+ channels in smooth muscle cells [132], cardiac myocytes [78], and modulate a variety of K^+ channels in neurons [176, 79]. A recent report suggests that there may be direct effects of arachidonate on gating of delayed rectifier channels. Cloned mouse $K_v1.2$ channels expressed in CHO cells display the delayed rectifier phenotype, but extracellular application of arachidonate speeds up activation and produces a time-dependent inactivation, presumably via open-channel block [116]. Inhibitors of cyclooxygenases or lipoxygenases did not alter this effect of arachidonic acid, presenting the possibility that receptor-mediated liberation of arachidonate may transiently alter the gating characteristics of K^+ channels which contribute to repolarization.

A number of other K^+ channels, including the muscarinic K^+ channel [29], the neuronal M-current [157, 16, 197] and the S-K^+ channel in *Aplysia* [26], are activated/modulated by arachidonic acid metabolites. In all of these examples, inhibition of arachidonic acid metabolism via either cyclooxygenase or lipoxygenase pathways eliminates the effect(s). The active arachidonic acid metabolite involved in stimulation of the muscarinic K^+ current is leukotriene C_4 [155, 156]. 12-lipoxygenase products (most likely 12-HETE and/or further metabolites) are implicated in the arachidonic acid-mediated effects on the neuronal M-current [197].

While the effects of arachidonate, fatty acids and phospholipids have been well-documented, the mechanism of action of these modulators on K^+ channels has not yet been established. Although direct interaction is always a possibility, the existence of intermediary proteins cannot at present be ruled out.

O_2-sensitive K^+ Channels

A class of K^+ channels unique to cell types which contain chemoreceptors for O_2 (including carotid body Type I (CB-1) cells and neuroepithelial body (NEB) cells from lung) are inhibited by hypoxia [1]. Both CB-1 and NEB cells contain an oxygen binding protein (NADPH oxidase), which is thought to translate alterations in O_2 tension into modulation of K^+ channel activity [48, 196]. Inhibition of NADPH oxidase with diphenylene iodonium (DPI) uncouples changes in O_2 tension from K^+ channel activity in NEB cells [196]. The K^+ channel involved in carotid body type I cells (CB-1 cells) has been well characterized at both the whole cell and single channel level, and has properties consistent with delayed rectifier K^+ channels [104, 49]. The mechanism of NADPH oxidase-mediated activation of the channel has not been identified, although it does not appear to involve a G protein-transduced pathway [48], and survives patch excision [72]. Definitive answers await the cloning of this K^+ channel from CB-1 or NEB cells, but the homology of K_v channel β1 subunits with enzymes in the aldo-keto reductase family [115, 28] make it interesting to speculate whether a K_v channel with an associated β subunit sensitive to redox state is involved.

G Protein-mediated K^+ Channel Activation/Modulation

The first ion channel to be identified as a direct G protein effector was the muscarinic K^+ channel of atrial myocytes [22, 139]. Since these initial observations, a variety of K^+ channels from both the inward rectifier (including K_{ATP}) [34, 80] and voltage-gated K^+ (including K_{Ca}) [175] channel families have been shown to be modulated by interactions with G proteins. Cloning of the muscarinic K^+ channel [86] and the intense interest in discerning the G protein subunit specificity for activation of this channel has made it a paradigm for the study of other channels similarly regulated. Gβγ subunits are the ''membrane-delimited'' activators of GIRK1 [101, 91], interacting with a binding site which has been localized to the C terminal domain [168, 148, 138, 32, 89]. Interaction of βγ subunits with GIRK1 leads to an increase in channel open probability, with a cooperativity suggesting binding of more than one βγ subunit per channel oligomer [92]. Elimination of the putative βγ binding domain [148] or substitution of the comparable region of a non-G protein-gated inward rectifier, BIRK10, eliminates the dependence of GIRK1 gating on βγ [138].

α-GTP is also involved in activation of K^+ channels, most notably K_{ATP} channels [70], large conductance K_{Ca} channels [173], and neuronal K^+ channels [178]. Many other K^+ channels types are potentially regulated by ''direct'' interactions with G protein subunits (i.e., activation occurs in excised patches in the presence of GTPγS without the need for any other second messenger), but the nature of the subunit (i.e., α versus βγ) has not been established. What remains to be established for any G protein-coupled K^+ channel is the mechanism by which interaction with G proteins confers an alteration in channel gating.

K^+ channels are also affected by the *ras* family of G proteins. *Ras* p21 plus GAP inhibits coupling of muscarinic receptors to atrial K^+ channels [193], while rap1A antagonizes this interaction [194]. *Ras*-mediated transformation of a number of cell types also affects K^+ channel expression, as discussed in a subsequent section.

Stretch and/or Flow-mediated Modulation of K^+ Channels

Activation or modulation of K^+ channels by membrane stretch and/or flow has been observed in neurons [122], renal proximal tubule [152], thick ascending limb [171], skeletal muscle [23], cardiac myocytes [140] and endothelial cells [130, 128]. The mechanism(s) of stretch/flow-mediated activation have not been identified, although membrane deformation has been proposed to directly influence channel gating. Further work is needed to define the role of the cytoskeleton and signaling cascades in the responses of individual K^+ channels types to stretch/flow.

Phosphorylation-dephosphorylation-mediated K^+ Channel Modulation

A ubiquitous mechanism for protein modulation is receptor-mediated phosphorylation-dephosphorylation. K^+ channels may require phosphorylation to function, or have their activity modulated by alterations in phosphorylation state. Phosphorylation increases the number of functional anomalous rectifier channels in *Aplysia* neurons [51]. Similarly, a K^+ cloned channel from rat brain requires PKA-mediated phosphorylation for channel function [186]. Phosphorylation can alter the gating properties of K^+ channels, as has been observed for cloned potassium channels from cardiac myocytes, where PKA-dependent phosphorylation increases both open probability and fractional occupancy of higher conductance states [63]. Phosphorylation may be either stimulatory (as in the above cases) or inhibitory, as exemplified by PKC-mediated inhibition of K_{ATP} channels in ventricular myocytes [99]; although fatty acid-sensitive PKC isozymes stimulate K_{ATP} channels [123]. A variety of K^+ channel types are sensitive to several protein kinase types, e.g., the *n* type K^+ channel in Jurkat T lymphocytes is stimulated by both PKA and PKC, although the effects are not additive and appear to modulate the same process [136]. In contrast, minK channels are activated by PKA [20] but inhibited by PKC [24]. In addition to PKA and PKC, many other protein kinases modulate K^+ channel activity, including myosine light chain kinase (MLCK) [6], tyrosine kinases [151, 62], Ca^{2+}/calmodulin-dependent protein kinase [131] and cGMP-dependent protein kinase [184]. Phosphorylation-mediated regulation via novel protein kinases is suspected for several K^+ channel types, since conventional protein kinase inhibitors do not block the modulatory responses mediated by Mg-ATP [77]. Protein kinases may be intimately associated with K^+ channels, as has been observed for maxi-K_{Ca} channels from mammalian brain [18]. The association of a protein kinase C-like activity survives K_{Ca} channel isolation and reconstitution into planar lipid bilayers [145], and may be accompanied by an associated phosphatase activity. When *Drosophila Slo* (maxi-K) channels are expressed in *Xenopus* oocytes, the activity in excised patches is altered by an associated protein kinase A, which can be blocked by a single serine mutation [39]. These results suggest that cytoplasmically localized protein kinases may also be intimately associated with K^+ channels, surviving both patch excision and vesicle isolation followed by planar bilayer reconstitution.

Molecular identification of various K^+ channels has begun to permit detailed investigations into the amino acid residue(s) modified by protein phosphorylation as well as the mechanisms of phosphorylation-induced alterations in K^+ channel activity. Some new insights are emerging. For instance, the rate of C-type inactivation of the $K_v1.3$ channel in oocytes is altered by patch excision, suggesting a potential requirement for phosphorylation. Mutation of up to 3 of 4 possible serine/threonine residues altered the rate of C-type inactivation but did not eliminate it [90]; rather, an extracellularly localized histidine residue was responsible. These results suggest caution when ascribing functional modulation directly to phosphorylation at particular residues.

The mechanism(s) of phosphorylation-induced alterations in channel gating are also being explored by mutagenesis. PKC eliminates rapid inactivation of an A-type K^+ channel ($K_v3.4$) via phosphorylation of two serine residues in the N terminal domain. Mutation of one of the serines to aspartic acid mimics the effects of PKC, suggesting that alterations in the net charge of the blocking particle affect inactivation [31]. Phosphorylation-induced alterations in charge density at the cytoplasmic face of delayed rectifier K^+ channels also alter channel gating, presumably by interactions between the phosphate group and the voltage sensor of the channel [137].

Regulation of K^+ Channel Expression

The most recently identified mechanism for regulation of K^+ channel activity is the regulation of expression of K^+ channels in response to a variety of stimuli. Expression of $K_v1.5$ channels is rapidly decreased upon membrane depolarization in pituitary cells [121, 97]. The 5′ untranslated region of the $K_v1.5$ gene contains a cAMP response element, and cAMP induces a rapid increase in $K_v1.5$ message level in cardiac myocytes, while in GH3 cells, similar treatment causes a decrease in $K_v1.5$ transcript [121]. cAMP can obviously have both direct effects on channel phosphorylation and on channel expression; this has been observed for $K_v1.1$ channels expressed in *Xenopus* oocytes [96]. Axons regulate the expression of Schwann cell K^+ channels via an as yet unidentified mechanism [27]. *Ras* transformation of fibroblasts induces expression of K_{Ca} channels [64]; trans-

formation of AtT20 cells (anterior pituitary-derived cells) with EJ-*ras* differentially affects expression of several K^+ channel mRNAs, resulting in the development of a more neuronal-like electrophysiological phenotype [55]. Cell depolarization or phorbol esters cause an immediate and significant increase in $K_v1.4$ transcripts in cardiac myocytes; differential regulation of $K_v1.4$ and $K_v1.5$ transcription was also observed in cardiac hypertrophy, suggesting dynamic regulation by various signaling cascades [110]. This area of K^+ channel regulation obviously needs further exploration.

Summary and Open Questions

From this brief summary of the range of modulators which affect plasma membrane K^+ channel activity, it is clear that we are at a transition point in our understanding of the functioning of K^+ channels. In vivo work has defined the vast range of modulatory factors which control net cellular K^+ permeability. Recent identification of many K^+ channel types at the molecular level has provided the foundation for determining the site(s) of modulator action within the K^+ channel structures, and will ultimately lead to an understanding of the mechanisms governing regulation of K^+ channel activities. There is an interplay between the structural and functional studies which has yielded some surprises with respect to predicted K^+ channel tertiary structure, including the assignment of the H5 or P loop to the permeation pathway of K_v channels, and the contribution of C terminal domains to the permeation pathway of IRK channels. Of considerable interest is the emerging paradigm of modular K^+ channel structures, i.e., a core permeability domain linked to an array of divergent C terminal domains which confer modulator-specific regulation. Determining the sites of C terminal modulatory domain interactions with the channel core will increase our understanding of the mechanism(s) by which modulation alters channel function.

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