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Ca^{2+}-Activated K^+ Channels: From Protein Complexes to Function

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Berkefeld H, Fakler B, Schulte U. Ca^{2+}-Activated K^+ Channels: From Protein Complexes to Function. *Physiol Rev* 90: 1437–1459, 2010; doi:10.1152/physrev.00049.2009.—Molecular research on ion channels has demonstrated that many of these integral membrane proteins associate with partner proteins, often versatile in their function, or even assemble into stable macromolecular complexes that ensure specificity and proper rate of the channel-mediated signal transduction. Calcium-activated potassium (K_{Ca}) channels that link excitability and intracellular calcium concentration are responsible for a wide variety of cellular processes ranging from regulation of smooth muscle tone to modulation of neurotransmission and control of neuronal firing pattern. Most of these functions are brought about by interaction of the channels' pore-forming subunits with distinct partner proteins. In this review we summarize recent insights into protein complexes associated with K_{Ca} channels as revealed by proteomic research and discuss the results available on structure and function of these complexes and on the underlying protein-protein interactions. Finally, the results are related to their significance for the function of K_{Ca} channels under cellular conditions.

I. INTRODUCTION

A. Ion Channel-Associated Protein Complexes

There is emerging evidence from functional analyses and proteomic research that the role of ion channels in cell physiology is not only determined by the pore-forming α-subunits but, as for many other classes of proteins, strongly depends on their molecular environment. The latter is formed by proteins that are either directly or indirectly associated with the α-subunits and that may modulate channel function (216), affect downstream signaling pathways (25, 31, 54), or shape spatio-temporal concentration gradients of ions or diffusible messengers (44, 109, 182). These molecular environments are often referred to as micro- or nanodomains, depending on their structural dimensions (5, 142), and are thought to represent a general principle for how membrane protein-based signaling is organized to guarantee specificity and proper rate of signal transduction. As a result, ion channels embedded in such entities can display diverse properties and functions depending on subcellular localization, developmental stage of the cell, and expression of distinct sets of partner proteins.

Within a given cellular proteome, formation and dynamic modulation of micro- or nanodomains depend on the affinity, kinetics, and specificity of the underlying protein-protein interactions. These biochemical parameters may vary over a broad range as a result of short-lived transitional states of enzymes modifying their protein substrates (1, 211), promiscuous and low-affinity binding to protein scaffolds allowing for local enrichment or co-localization of proteins (90), or constitutive and stable quaternary protein structures (8, 42, 45). This spectrum

sets the framework for the observed specificity and time scales of individual signaling events and their dynamic changes. Constitutively associated proteins are able to mediate effects very rapidly through allosteric interactions compared with interactions involving diffusion of protein partners. Thus formation of stable complexes with auxiliary subunits is typically observed with ion channels involved in rapid electrical signaling (185).

It should be noted that the term *protein complex* is often used in different contexts or for various types of protein-protein interactions. We prefer a more stringent definition in that physiological protein complexes are assemblies of proteins *1*) with defined (saturable) stoichiometry, *2*) kinetic stability that allows their direct observation or isolation, and *3*) that are actually present in a native biological system. Accordingly, constituents of ion channels not involved in pore formation but affecting channel function are referred to as auxiliary/accessory subunits, while larger functional units made up from stable assemblies of distinct (and functionally independent) complexes are termed supercomplexes (as proposed by Schägger and Pfeiffer, Ref. 183).

B. Proteomic Analysis of Protein Complexes

Of the methods available for identification of protein interactions (185), only a few are capable of directly identifying protein complexes in natural source material. Biochemical copurification of protein interaction partners coupled to sensitive mass spectrometric analysis, referred to as "functional proteomics," is the current method of choice (63) and has been implemented in ion channel research (185).

The success of this strategy critically depends on two factors. The first is the ability to preserve the integrity of protein complexes during their isolation from native tissues. Although many soluble complexes can be stably isolated as shown in systematic studies (61, 77), this is more challenging for complexes associated with membrane proteins as they require solubilization with detergents that may actually disrupt the underlying protein-protein interactions. Consequently, stability and integrity of complexes must be carefully monitored by techniques such as native PAGE analysis (183) to optimize purification of native protein complexes. The second factor is specific enrichment of the target protein complex that is generally done by affinity purification using immobilized antibodies directed against individual target proteins. Although high specific enrichment can be achieved in a single step, antibodies often present with individual and "unpredictable" properties that give rise to common artifacts. These include direct and indirect binding of proteins independent from the target (cross-reactivity), selection biases towards target protein isoforms, modifications or complex subpopulations, or even disruption of target complexes. Strategies are required to eliminate the resulting errors: proteins copurified independent of the target (false positives) can be identified through comparison with purifications from target knockout material using the same antibody; and miss of interaction partners due to selection biases or complex disruption (false negatives) can be reduced by combining affinity purifications with multiple antibodies targeting different epitopes. Finally, liquid chromatography-coupled mass spectrometry (LC-MS/MS) plays an essential role in this approach as the only technique providing unbiased information on the identity and quantity of the isolated proteins (63). The mentioned techniques and strategies have proven successful in a number of functional proteomic studies on ion channel-associated protein complexes (12, 116, 139, 186, 189, 251).

C. Characterization of Ion Channel-Associated Complexes In Vivo

The proteomic approach outlined above provides information on the composition of protein (super)complexes including identity and amount of their constituents, but it does not (per se) give insights into the stoichiometry and distribution of complexes over the various subcellular compartments, nor does it provide information on the stability of complexes under cellular conditions or the functional significance of the underlying protein-protein interactions. Such data may be obtained by in vivo studies using optical and/or electrophysiological methods on native as well as heterologously reconstituted protein (super)complexes (11, 12, 109, 182).

These techniques complement the biochemical results in three ways: *1*) they can provide independent evidence for protein assembly into complexes, *2*) give information about the dynamics of protein complexes in living cells, and *3*) can be used to analyze distance relationships between partners. When labeled with suitable fluorophores, colocalization of proteins in specific cellular compartments can be confirmed. Although this is not compelling evidence for a biochemical interaction due to a relatively poor spatial resolution in the range of ~200 nm (60), analysis of the covariation between spatial distributions of pairs of fluorescence-labeled proteins has been used as a measure for interaction probability (110, 219). More defined information on molecular distances can be obtained by Förster resonance energy transfer (FRET) that occurs between an excited donor and an acceptor chromophore [typically distinct fluorescent proteins, mostly green fluorescent protein (GFP) and variants thereof, fused to complex constituents] if located within ~10 nm of each other (223).

Functional effects of partner proteins associated with ion channels have been studied in electrophysiolog-

ical recordings, mostly in patch-clamp configurations that allow for excellent signal-to-noise ratio and time resolution together with direct manipulations of the underlying protein-protein interactions or for changes of the channels' nano-environments. Thus defined Ca^{2+} concentration gradients established around Ca^{2+} sources by mobile buffers such as EGTA and BAPTA were used to determine the distances between these sources and Ca^{2+}-activated potassium channels (see below) (50). Similarly, application of agonists/inhibitors of protein activities have revealed the functional relevance of associated partners for the molecular architecture and the cell physiology of ion channel complexes (47, 82, 86, 118, 214).

D. Overview on Calcium-Activated K^+ Channels

Ca^{2+}-activated potassium (K_{Ca}) channels are widely expressed in neuronal and nonneuronal tissues including epithelia, smooth muscle, and sensory cells where they couple membrane potential and intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) (53, 163, 222). Activated upon an increase in $[Ca^{2+}]_i$, K_{Ca} channels give rise to an efflux of K^+ which via re/hyperpolarization of the membrane potential feeds back onto $[Ca^{2+}]_i$ by limiting Ca^{2+} influx either through deactivation of voltage-gated calcium (Cav) channels or through increased transport activity of Na^+/Ca^{2+} exchangers. Accordingly, K_{Ca} channels shape the amplitude and duration of Ca^{2+} transients and thus affect the downstream signaling pathways that are triggered by changes in $[Ca^{2+}]_i$ (50).

Based on their biophysical properties, K_{Ca} channels have been classified into two subtypes: one, exhibiting large unitary conductance and gated by the cooperative action of membrane depolarization and $[Ca^{2+}]_i$, is termed BK_{Ca}, and the other, displaying small conductance and gated solely by $[Ca^{2+}]_i$, is referred to as SK_{Ca} (180).

Both types of K_{Ca} channels have been implicated in a variety of physiological processes, which for BK_{Ca} range from regulation of smooth muscle tone (18, 132) and microbial killing in leukocytes (2) to modulation of hormone and neurotransmitter release (228, 245). In central neurons, where they exhibit a broad expression pattern throughout most regions of the brain, BK_{Ca} channels contribute to repolarization of action potentials (APs) (197), mediate the fast phase of afterhyperpolarization following an AP (70, 103, 236), shape the dendritic Ca^{2+} spikes (66), and influence the release of neurotransmitters (245). Similarly, the cell physiology of SK_{Ca} channels covers a wide range from controlling uterine contractility (22) and vascular tone (210), modulation of hormone secretion (46, 208, 250), control of cell volume in red blood cells (78, 220), activation of microglia and lymphocytes (52, 85, 91) to regulation of excitability, firing pattern, and synaptic signal transduction in central neurons (24, 73, 112, 143, 194, 234). In addition, SK_{Ca} channels together with nicotinic acetylcholine receptors reconstitute an unusual inhibitory synapse in auditory outer hair cells, where the excitatory transmitter acetylcholine drives an inhibitory K^+ conductance (64, 149, 246). As aforementioned in a more general sense, these functions are not operated by the α-subunits of K_{Ca} channels as stand-alone units, but rather result from their integration into protein (super)complexes (17, 38, 107).

This review is intended to align biochemical with functional evidence for the existence of such K_{Ca} channel-associated protein complexes in vertebrates. We will focus on defined BK_{Ca} and SK_{Ca} channel (super)complexes that have been thoroughly investigated with respect to both biochemistry and function as put forward above, rather than providing a comprehensive list of suggested interaction partners as done previously (120). Moreover, we will discuss the molecular dimensions of these complexes, advanced methods for their characterization, and implications for their role in cell physiology.

II. MOLECULAR PARTNERS OF BK_{Ca} CHANNELS

The BK_{Ca} channel core is a tetrameric assembly of α-subunits (BKα), polypeptides of 125–140 kDa encoded by a single gene (termed Slo or maxiK, nomenclature according to IUPHAR is KCNMA1) that may be spliced at several sites (244). Unique among K^+ channels, BKα comprises seven transmembrane domains (S0-S6) placing the short NH_2 terminus extracellularly and the large COOH terminus, roughly two-thirds of the protein, at the intracellular side of the membrane (Fig. 1*A*) (129). This intracellular domain contains four hydrophobic segments (S7-S10), two regulating conductance of K^+ (RCK) domains, and a stretch of aspartate residues that are known as the "Ca^{2+} bowl" (120). Tertiary folding of these domains reconstitutes a binding site(s) for Ca^{2+} with micromolar affinities (K_D of ~10 μM) whose occupancy provides one source of energy for channel opening (7, 123, 184, 206, 243). The other is membrane depolarization that is fed into the channel gating by movement of the voltage-sensing segment S2–4 (79, 80, 122, 153). Both stimuli, Ca^{2+} binding and membrane depolarization, converge allosterically on the gating machinery that is experimentally visualized by a shift of the voltage-dependent activation curve towards hyperpolarized potentials in response to increasing Ca^{2+} concentrations (34, 104, 179, 209). Under cellular conditions, BK_{Ca} channels are usually operated by both stimuli in a concerted action, with robust activation in the physiological voltage range requiring $[Ca^{2+}]_i$ of $\geq$10 μM (37, 105, 127). In rare cases, BK_{Ca} channels were shown to operate at lower $[Ca^{2+}]_i$ (162, 163) or even in the absence of intracellular Ca^{2+} (62, 212).

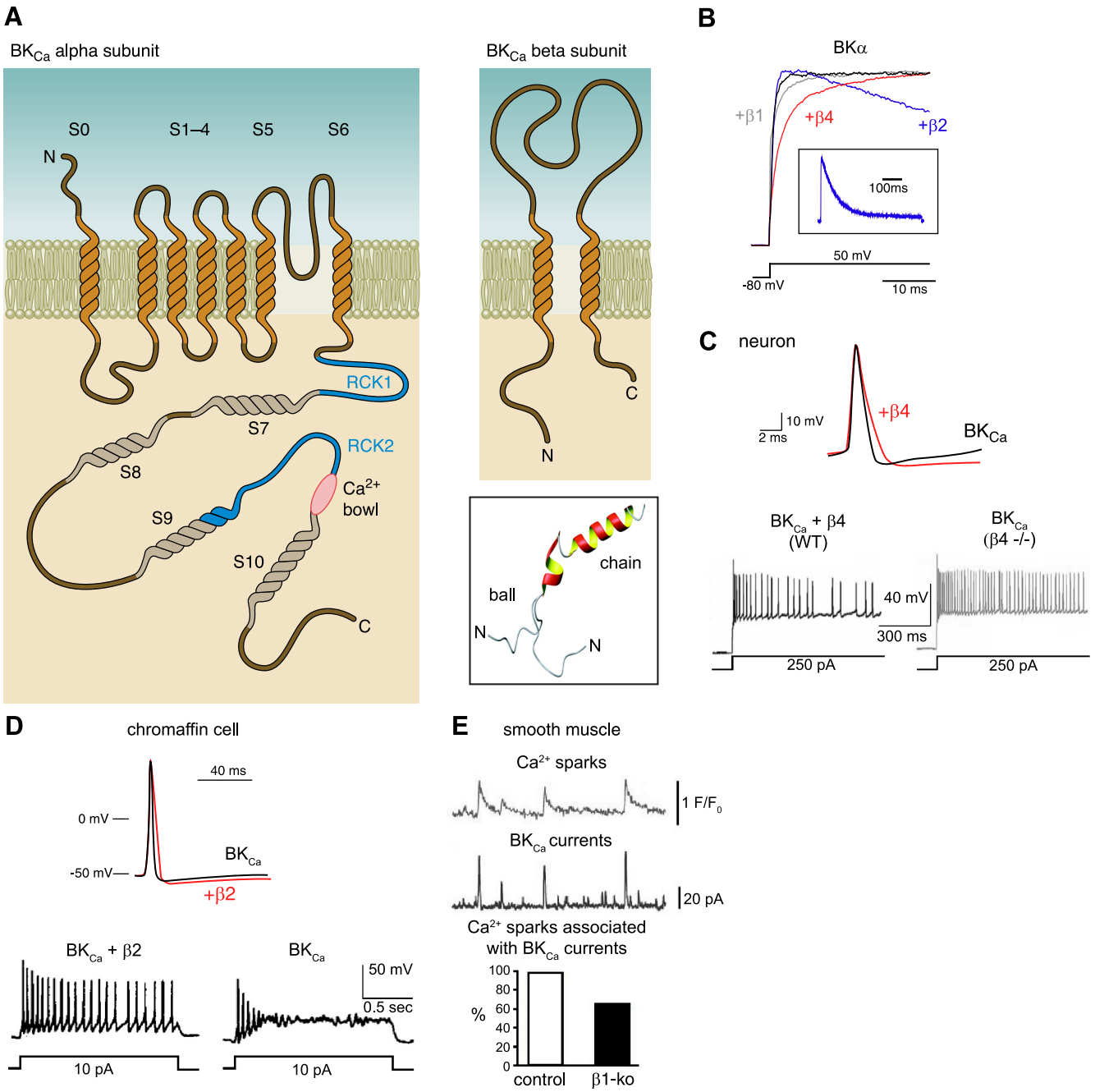


FIG. 1. The BKβ subunits shape the properties of BK_{Ca} channels. *A*: membrane topology of BKα and BKβ as described in the text. *Inset*: structure of the NH_2-terminal "ball-and-chain" domain of BKβ2 as derived from solution NMR (10). *B*: K^+ currents through BK_{Ca} channels assembled either from BKα alone or from BKα and the indicated BKβ subunits upon heterologous expression in *Xenopus laevis* oocytes. *Inset*: step response of BKα-BKβ2 channels on a compressed time scale. *C–E*: significance of BKβ subunits for the physiological function of BK_{Ca} channels. *C*: BKβ4 reduces the firing rate of dentate gyrus granule cells by action potential (AP) broadening and prolongation of the fast afterhyperpolarization (fAHP). [Modified from Brenner et al. (19).] *D*: BKβ2 promotes tonic firing in chromaffin cells. [Modified from Solaro et al. (193).] *E*: BKβ1 ensures coupling of BK_{Ca} channels to Ca^{2+} sparks in arterial smooth muscle. [Modified from Brenner et al. (21).]

A. Auxiliary β-Subunits of BK_{Ca}

1. *Biochemistry and structure*

Although tetramers of BKα are functional, the vast majority of BK_{Ca} channels present in the plasma membrane of vertebrate cells are associated with auxiliary β-subunits (BKβ); in contrast, no BKβ was identified in invertebrates (12, 150). At present, four genes coding for BK_{Ca} β-subunits (termed KCNMB1–4) are known, with BKβ3 giving rise to four distinct isoforms as a result of alternative splicing (120, 150, 216). BKβ1, the first β-sub-

unit in hand, was identified as a noncovalently attached constitutive partner of BKα in experiments purifying BK_{Ca} channels from smooth muscle membranes following toxin cross-linking (97). Subsequent database searches revealed the homologous proteins BKβ2–4 [β2 (225, 240), β3 (20, 217, 241), and β4 (229)] that exhibit sequence homology of >45% with BKβ1 and all share an overall topology with two transmembrane segments flanking a large, glycosylated extracellular loop and short intracellular NH_2 and COOH termini (Fig. 1*A*) (96, 150, 216). Interaction of BKα and BKβ involves multiple contact sites: the first transmembrane domain of BKβ touches S1 and S2 of BKα, and the extracellular extension of the second transmembrane domain of BKβ contacts the S0 segment of BKα (115, 237).

Although the rotational symmetry of the BKα tetramer suggests association with up to four β-subunits, as originally proposed by Knaus et al. (97), the α-β stoichiometry is still controversially discussed. In heterologous systems, titration effects on the α/β ratio (up to 1/1) were observed (41, 227), but their relevance for the complex stoichiometry in native tissue is still unclear (89, 227). Functional measurements and toxin binding studies suggest that in most tissues the majority of BK_{Ca} channels are intimately associated with at least one of the BKβ proteins (209, 215, 239).

2. Modulation of channel gating

The impact of the coassembly with β-subunits on the gating of BK_{Ca} channels has been extensively reviewed (150, 181, 216), documenting effects on gating, pharmacology, as well as on trafficking mostly in a β-subtype-specific manner. The following effects of BKβs on the pore properties gating and permeation may be summarized (Fig. 1*B*).

A) ACTIVATION/DEACTIVATION. The BKβ subunits alter the activation characteristics of BK_{Ca} channels by affecting both the Ca^{2+} and voltage dependence of opening and closing transitions (Fig. 1*B*). As the best characterized example, association with BKβ1 strongly increases the apparent Ca^{2+} sensitivity of BK_{Ca} channels, likely by an allosteric mechanism that lowers the energy of the open state (205). As a consequence, channel opening occurs at more negative potentials and with faster kinetics (7), whereas deactivation is slowed down. Structurally, this effect has been attributed to the intracellular domains of BKβ1 (151) and the S0 helix of the BKα subunit (224). Association with BKβ4 leads to a deceleration of both the activation and deactivation time courses by a yet unclear mechanism(s) (71, 229; Fig. 1*B*).

B) INACTIVATION. BKβs 2 and 3 both endow BK_{Ca} channels with rapid inactivation through a classical "ball-and-chain" mechanism where a partially folded NH_2-terminal domain tethered to the first transmembrane segment via a helical chain domain plugs the open pore from the cytoplasmic side (Fig. 1*A*) (10, 113). While the BKβ2 subunit promotes complete inactivation (Fig. 1*B*), the three splice variants of BKβ3 (β3a-c) only mediate an incomplete channel block (113, 217).

C) PERMEATION. Association with the BKβ subunits 2 and 3 induces voltage-dependent rectification of the BK_{Ca}-mediated K^+ currents through a cluster of positively charged amino acids in the cysteine-rich extracellular loop connecting the two transmembrane domains that acts as an additional extracellular gate (20, 28, 241, 249).

3. Effects on channel pharmacology

The scorpion toxins charybdotoxin (ChTX) (133) and iberiotoxin (IbTX) (58) are peptide blockers of BK_{Ca} channels that bind to the outer vestibule of the pore with nanomolar affinity. As shown in cross-linking experiments, the toxin binding sites include part of the extracellular loop of the BKβ subunits (96). Distinct structure and charge distribution in this region determine the differential effects of the BKβs on toxin binding (28, 248). Thus, while coassembly with BKβ4 or BKβ2/3 strongly reduces ChTX-mediated inhibition of BK_{Ca} channels by 1,000- and 30-fold, respectively (59, 130, 240), channels associated with BKβ1 retain their high sensitivity to this blocker (74).

In addition to peptide toxins, BKβs also modify the effect of several metabolites and small molecule drugs on BK_{Ca} channel activity. However, as the binding sites for these ligands have not been determined, it remains unclear whether BKβ subunits actually act as (co)receptors or induce allosteric changes in BKα. The effect of unsaturated fatty acids such as arachidonic acid is best understood. They counteract rapid inactivation conferred by BKβ2 and BKβ3 subunits (203). Furthermore, steroid hormones have been described to increase BK_{Ca} channel activity in a BKβ-subtype-dependent manner, although at concentrations beyond the levels assumed to occur under physiological conditions (9, 95, 135, 218).

4. Effects on protein processing/trafficking

As for auxiliary subunits of other ion channels, BKβ subunits were shown to influence trafficking of BK_{Ca} channels to the plasma membrane, although in a distinct manner. While most β-subunits of ion channels promote surface expression (43), BKβ2 and BKβ4 actually decrease the number of BK_{Ca} channels in the cell membrane (229, 247); reports on the effects of BKβ1 on trafficking are conflicting (94, 215).

5. Implications for cell physiology

The BKβ subunits exhibit quite distinct expression profiles as revealed by in situ hybridization as well as by

Northern and Western blot analysis (9, 96, 225). While BKβ1 predominates in smooth muscle (87), BKβ4 is primarily found in neuronal tissues (9, 20). BKβs 2 and 3 exhibit a more diverse pattern, with BKβ2 displaying robust expression in ovary, adrenal gland, brain, and heart (20, 225) and BKβ3 found in various organs including adrenal gland, pancreas, and heart (9, 217, 240, 241).

In some of these tissues, efforts have been made to link cell-type specific composition of BK_{Ca} channels to distinct physiological functions.

1) In smooth muscles, BK_{Ca} channels are activated either by Ca^{2+} sparks, Ca^{2+} puffs released from intracellular stores, and/or by L-type Cav channels (21, 147, 160). In each case, BK_{Ca}-mediated hyperpolarization counteracts further Ca^{2+} flux by deactivation of Cav channels and thus promotes relaxation of the muscle cell (84). Targeted deletion of the KCNMB1 gene confirmed the specific role of the BKβ1 protein in vascular and tracheal smooth muscle (Fig. 1*E*): the resulting BKβ1-deprived BK_{Ca} channels exhibited decreased Ca^{2+} sensitivity, reducing the coupling between Ca^{2+} sparks and BK_{Ca} channel activity (21, 164, 166, 190). As a consequence, the vasoregulatory mechanisms were impaired, which led to increased vasoconstriction of cerebral arteries and elevated blood pressure levels (reviewed in Ref. 155).

2) In the adrenal gland, two types of chromaffin cells have been distinguished based on their distinct BK_{Ca} currents (Fig. 1*D*): one type displaying noninactivating and rapidly deactivating K^+ currents as mediated by BK_{Ca} channels assembled from BKα only, and another type with inactivating and slowly deactivating currents as known for BK_{Ca} channels associated with BKβ2 or BKβ3 (150, 240). As a result of these distinct gating properties, the respective BK_{Ca} channels enforce different firing patterns on the chromaffin cells. The slowly deactivating BKα-BKβ2/3 channels give rise to a pronounced afterhyperpolarization that relieves voltage-dependent Na^+ channels from inactivation and promotes repetitive or tonic firing (150). In contrast, the rapidly deactivating channels lead to only small afterhyperpolarizations that promote firing at a more phasic pattern (193).

3) In central nervous system (CNS) neurons, BK_{Ca} channels contribute to repolarization of APs and give rise to a fast afterhyperpolarization (fAHP) which both impact on neuronal firing by "spike sharpening" depending on the properties of the BK_{Ca}-mediated K^+ currents (11, 197). In hippocampal pyramidal cells, inactivating BK_{Ca} channels, presumably composed of BKα and BKβ2, promote frequency-dependent AP broadening along a spike train (191). This phenomenon results from successive inactivation of BK_{Ca} channels during a train of APs when the frequency of the AP-triggered channel activation exceeds the rate of recovery from inactivation. In hippocampal granule cells, the slowed activation kinetics induced by coassembly with BKβ4 appears to operate as a "low-pass filter" that prevents high-frequency firing and spike sharpening as seen in mice with a targeted deletion of this β-subunit (Fig. 1*C*). Removal of this filtering function may be responsible for increased susceptibility to temporal lobe seizures observed with the BKβ4 knock-out animals (19).

4) In auditory sensory hair cells of amphibians, birds, and fish, BK_{Ca} channels participate in "electrical ringing," a resonance phenomenon fundamental for hearing in these animals (4, 35, 56, 57, 108, 202). Basically, electrical ringing is depolarization-repolarization cycles that are generated by serial and repetitive activation of L-type Cav channels and BK_{Ca} channels (238). The frequency of these electrical oscillations is determined by the amplitude and kinetics of the BK_{Ca} currents and varies between hair cells along the axis of the hearing organ as a result of an expression gradient of the BKβ1 subunit (53, 171, 172). Thus cells expressing BK_{Ca} with minor or no assembly with this β-subunit activate rapidly and enable oscillations at high frequencies, while increasing association with BKβ1 slows BK_{Ca} channel deactivation and increases Ca^{2+} sensitivity. Both factors promote sustained activity and suppression of high-frequency oscillations (171).

B. Complexes of BK_{Ca} and Cav Channels

In central neurons, the Ca^{2+} ions required for activation of BK_{Ca} channels are mainly delivered by Cav channels as blocking these channels effectively inhibited the respective K^+ currents (48, 65, 103, 168). In fact, subtype-specific peptide toxins or reagents identified a subset of the Cav channel family as the major Ca^{2+} sources fueling BK_{Ca} (26). In addition, the robust activation of BK_{Ca} currents observed in neurons upon physiological voltage stimuli necessitated $[Ca^{2+}]_i$ to be in the range of $\geq$10 μM (12). As such Ca^{2+} concentrations are thought to be restricted to the immediate vicinity of active Ca^{2+} sources (5, 142), these results implied close colocalization of BK_{Ca} and Cav channels. The molecular mechanism linking both types of channels has recently been resolved as a direct channel-channel interaction (12).

1. Biochemistry of BK_{Ca}-Cav interaction

First evidence for a biochemical link between BK_{Ca} and Cav channels was obtained by Grunnet and Kaufmann (69) demonstrating coprecipitation of BKα and Cav1.2 and Cav1.3, both encoding distinct α-subunits of L-type channels. A comprehensive proteomic approach using multiple affinity purifications in combination with high-resolution quantitative mass spectrometric analysis identified the set of BK_{Ca}-associated Ca^{2+} sources in the rodent brain. Accordingly, BK_{Ca} channels from mouse and rat brain assembled from BKα and BKβ subunits 2 and 4 were found to be tightly associated with the Cav subtypes Cav1.2 (L-type channels), Cav2.1 (P/Q-type channels), and

Cav2.2 (N-type channels) that effectively copurified with the BK_{Ca} channels together with their auxiliary β-subunits Cavβ1–3 (12). Reverse purification of these Cav channel subtypes, control experiments using brains with targeted knock-outs, and copurifications from heterologous expression systems (12 and unpublished results) confirmed the formation of channel-channel supercomplexes of BK_{Ca} and Cav channels. The intimate association of both channels was found to occur through direct interaction of their pore-forming α-subunits (12) presumably via contacts between parts of their transmembrane domains (unpublished results; Fig. 2).

When gently solubilized from brain membranes, BK_{Ca} channel complexes displayed high molecular masses ranging from ~0.8 kDa, corresponding to the size of hetero-octamers composed of 4 BKα and 4 BKβ subunits, up to ~1.8 MDa (12), a size that would be expected for an assembly of such hetero-octameric BK_{Ca} channels with 4 Cav channels. Figure 2 depicts a structural model generated for such a BK_{Ca}-Cav supercomplex on the basis of crystallographic data and homology modeling (see legend for details). The pore-to-pore distance in this compact arrangement is ~10 nm.

2. *Functional analysis of synthetic BK_{Ca}-Cav complexes*

BK_{Ca}-Cav supercomplexes could be readily reconstituted in heterologous systems, such as cultured cells and *Xenopus* oocytes, where they assembled upon expression of the respective α- and β-subunits as visualized by patch-clamp experiments (Fig. 3*A*) (12). In inside-out patches with EGTA buffering Ca^{2+} on the cytoplasmic side, the reconstituted BK_{Ca}-Cav complexes displayed a biphasic current output in response to step depolarizations exceeding the activation threshold of the Cav channel: an initial inward current carried by Ca^{2+} was followed by an outward K^+ current reflecting the Cav channel fueling the coassembled BK_{Ca} channel (Fig. 3, *A* and *B*). This functional coupling was insensitive to EGTA even at high millimolar concentrations, but could be disrupted by BAPTA, a Ca^{2+} chelator with 100-fold faster binding kinetics (141). In addition, calibration measurements of the activation kinetics at defined $[Ca^{2+}]_i$ estimated the effective Ca^{2+} concentration seen by BK_{Ca} channels within BK_{Ca}-Cav supercomplexes to values $\geq$10 μM (12). Together with the $[Ca^{2+}]_i$ profiles generated by EGTA and BAPTA (5, 50), these functional measurements placed both channels within ~10 nm of each other and thus provided independent support for the structural model derived from biochemistry and proteomic analyses (Fig. 2).

The resistance of channel-channel coupling to interference with EGTA proved also useful for probing the specificity of the BK_{Ca}-Cav coassembly. While supercomplexes with Cav2.1, Cav2.2, and Cav1.2 were insensitive to EGTA, Cav2.3 channels failed to activate K^+ currents through the coexpressed BK_{Ca} channels under these conditions (12). This confirmed the results from the proteomic analyses and demonstrated that formation of BK_{Ca}-Cav supercomplexes is restricted to a subset of the Cav channel family.

Another interesting feature evolving from the functional recordings with synthetic BK_{Ca}-Cav complexes is the tight kinetic control exerted by the Cav channel on its BK_{Ca} partner channel. Thus current outputs of either BK_{Ca}-Cav2.1 or BK_{Ca}-Cav1.2 complexes revealed that the distinct activation characteristics of the two Cav subtypes were translated into K^+ currents with distinct voltage dependence as well as distinct time courses. More explicitly, BK_{Ca} channels activated faster and at more negative

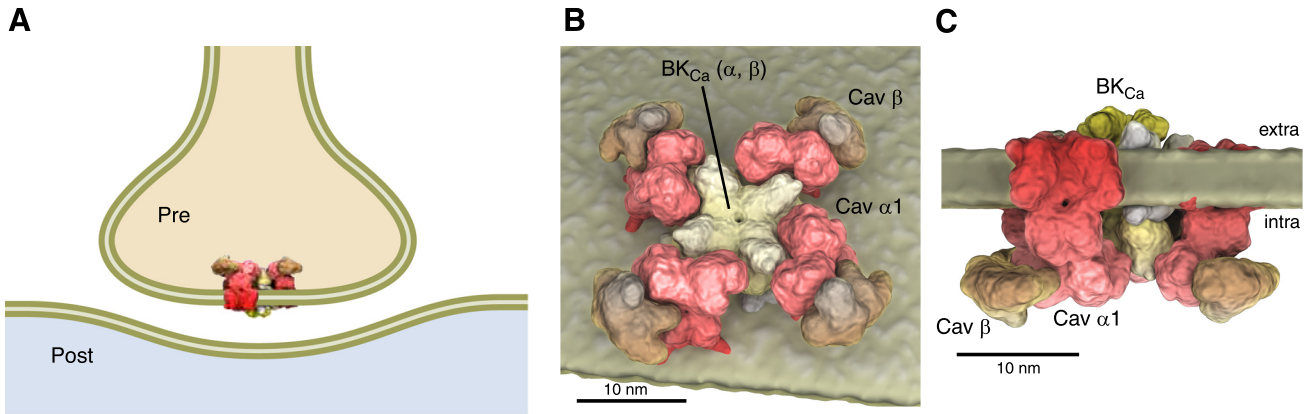


FIG. 2. Structural model of a BK_{Ca}-Cav channel supercomplex. Top view (from cytoplasmic side; *B*) and side view (*C*) of a space-filling model of a supercomplex assembled from BK_{Ca} (light yellow, green) and 4 Cav channels (red and brown) in a presynaptic bouton (*A*). The model was generated with the Maya platform (Autodesk Maya 3D; Ref. 207) using database entries for Kv1.2 (pdb entry 2A79), MthK (1LNQ), RCK domains of MthK (2AEF), and Cavβ3 (1T3L) together with molecular modeling; scale bar is 10 nm. This model is in very close agreement with the recently resolved cryo-electron microscopic structure of a BKα tetramer (226).

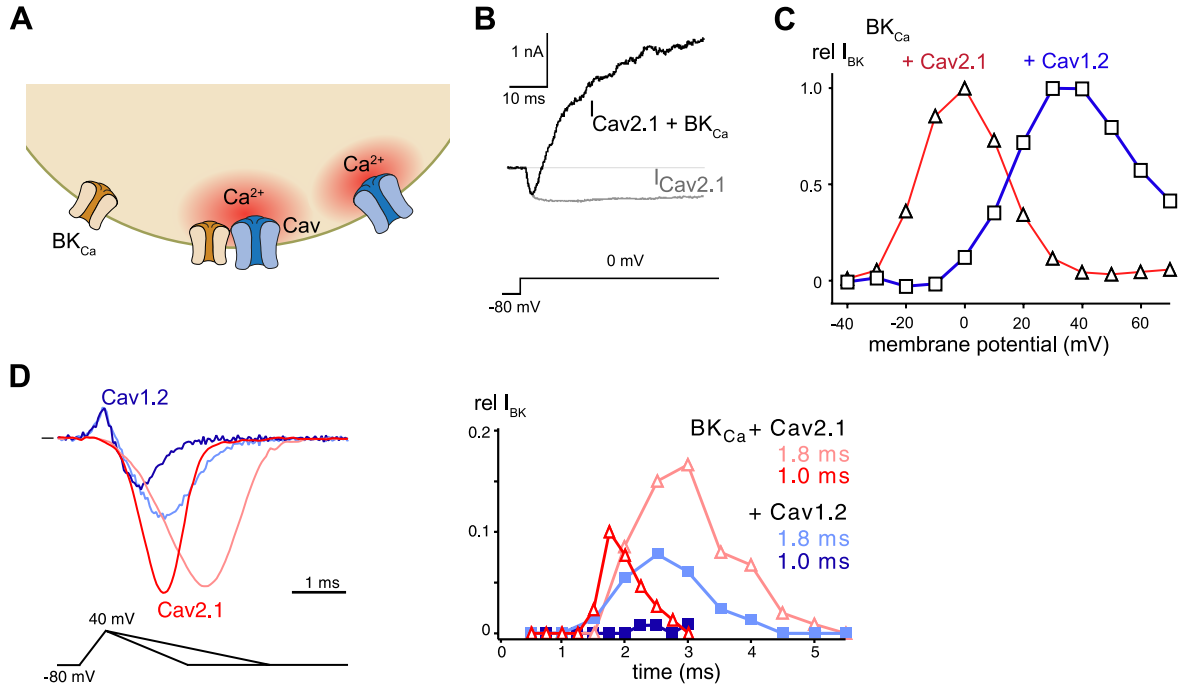


FIG. 3. Current output of BK_{Ca}-Cav complexes are determined by their Cav subunit(s). *A*: scheme illustrating assembly of BK_{Ca}-Cav complexes in heterologous expression systems together with profiles for $[Ca^{2+}]_i$ (spatial gradients in red). *B*: current output of a BK_{Ca}-Cav2.1 complex heterologously reconstituted in *Xenopus* oocytes in response to the indicated voltage step. Note that outward K^+ currents are preceded by a Ca^{2+} inward current (gray trace, recorded with Cav2.1 channels alone). *C*: current-voltage relation of the K^+ currents through BK_{Ca} channels depends on the coassembled Cav channel subtype. *D*: Ca^{2+} current responses of Cav1.2 and Cav2.1 channels (*left*, *top*) and K^+ current response of BK_{Ca}-Cav1.2 and BK_{Ca}-Cav2.1 complexes (*right*) recorded upon AP-like stimuli (*left*, *bottom*) with half-widths of 1 and 1.8 ms. [From Berkefeld and Fakler (11).]

voltages when associated with Cav2.1 than with Cav1.2, directly reflecting the differences in activation characteristics between the two Cav channel subtypes (Fig. 3*C*). The respective complexes also differed considerably in their responses to AP-like voltage stimuli (Fig. 3*D*). While Cav2.1 channels delivered significant Ca^{2+} currents even with short APs, Cav1.2 responded more prominently to longer-lasting APs. Likewise, BK_{Ca} channels were already activated during short APs when integrated into complexes with Cav2.1 channels, whereas their activation in complexes with Cav1.2 required a markedly longer AP duration (Fig. 3*D*).

Together, the properties of the synthetic BK_{Ca}-Cav complexes revealed that direct coassembly of these channels *1*) guarantees reliable and robust activation of BK_{Ca} in the physiological voltage range and even in the presence of highly active Ca^{2+} buffering systems, and that *2*) the complexes readily translate local Ca^{2+} influx into membrane hyperpolarizations which can be effectively tuned by the respective Cav subtype.

3. *Functional analysis of native BK_{Ca}-Cav complexes*

The functional properties of heterologously reconstituted BK_{Ca}-Cav complexes as reviewed above perfectly parallel those described for the coupling between both channel types in native cells: differential effects of the chelators EGTA and BAPTA on the channel-channel coupling were obtained in hippocampal pyramidal cells (103, 126, 198, 221), dentate gyrus granule cells (19), striatal cholinergic interneurons (65), frog saccular hair cells (175, 176), the frog neuromuscular junction (177), and chromaffin cells (167, 169). The functional variability of BK_{Ca} channel activity generated by coassembly with the distinct Cav channel partners manifests as tissue-specific diversification generated by the expression pattern of these Cav channels. Thus P/Q-type and N-type channels are predominantly found in the pre- and postsynaptic compartment of neuronal cells, as demonstrated for cholinergic interneurons, saccular hair cells, or cerebellar Purkinje cells (48, 65, 101, 175, 176), whereas L-type channels are mostly localized to cell somata and dendrites of hippocampal pyramidal cells, in frog saccular hair cells or chromaffin cells (103, 145, 173, 197, 198).

A property not yet analyzed in heterologous coexpressions of BK_{Ca} and Cav channels is the efficiency of complex formation. However, this question has been pursued in a study where Sun and Grinnell compared BK_{Ca} activity triggered by Cav channels and that triggered by exogenous Ca^{2+} in patches excised from motor nerve terminals of *Xenopus laevis* (204). Interestingly, these authors found an efficiency for complex formation of close to 80% at the outer membrane of the varicosities,

while it was almost 100% at the release face of the presynapse. This fits with the observation of different BK_{Ca} complex populations in native gel separations (12) and suggests that formation of BK_{Ca}-Cav supercomplexes may be intrinsically dynamic or requires yet unidentified factors promoting or restricting their assembly.

4. Implications for cell physiology

For cellular physiology, formation of BK_{Ca}-Cav complexes provides a straightforward and favorable molecular solution to several problems. First, activation of BK_{Ca} channels becomes virtually independent of the global cellular Ca^{2+} environment (126, 136, 175), but rather requires very local delivery of micromolar $[Ca^{2+}]_i$ that should not affect other Ca^{2+}-dependent processes. Second, the spatio-temporal restriction of Ca^{2+} signaling allows for signaling at increased frequencies, at the same time minimizing energy consumption and potentially detrimental effects of increased $[Ca^{2+}]_i$ (128, 136). Finally, selective coupling of Cav channels to BK_{Ca} is an important mechanism to ensure specificity of Ca^{2+}-mediated signaling.

In neurons, BK_{Ca} currents are activated during the repolarization phase of an AP and contribute to its time course as visualized by either blocking the BK_{Ca} or the Cav subunit of the BK_{Ca}-Cav complexes (Fig. 4*A*). The amplitude and time course of the BK_{Ca} currents, and consequently their impact on AP repolarization, strongly depend on the Cav channel subtype coassembled with BK_{Ca}, very similar to what was described above for the synthetic BK_{Ca}-Cav complexes (Fig. 3*D*). This fine-tuning is believed to underlie tissue-specific functions; for example, in cerebellar Purkinje cells that display short APs (48, 235) allowing for higher firing rates, BK_{Ca} channels were found to be predominantly fueled by Cav2.1 channels, while broad APs like in hormone-secreting chromaffin cells involve both Cav1.2 and Cav2.1 channels (168).

In addition to AP repolarization, the K^+ current output of BK_{Ca}-Cav complexes in presynaptic terminals was shown to feed back onto the intracellular Ca^{2+} profile, thus influencing synaptic transmission in two distinct ways (Fig. 4, *B* and *C*). In CA3-CA3 synapses and frog neuromuscular junctions, the BK_{Ca}-mediated K^+ currents shortened the period of Ca^{2+} influx and, as a consequence of this negative feedback, reduced the release of transmitters and the amplitude of the postsynaptic potential (Fig. 4*B*) (68, 170, 177). In contrast, Pattillo et al. (156) reported a positive feedback of the BK_{Ca}-Cav complexes on the synaptic transmission in frog nerve-muscular synapses. According to this report, the BK_{Ca}-mediated re/hyperpolarization increased the driving force for Ca^{2+} influx, thus leading to increased postsynaptic currents (Fig. 4*C*). These differential effects might be explained by the distinct timing between the BK_{Ca}-carried K^+ conductance and the release-triggering Ca^{2+} conductance (156) as is expected for BK_{Ca}-Cav complexes with distinct subunit composition.

C. Further Interactors of BK_{Ca} Channels

The stringent criteria put forth in section I*D* were barely met by other proteins implicated as candidate interactors of BK_{Ca} channels (for a rather extensive list, see Ref. 120). For some of these proteins, interaction with BK_{Ca} lacks validation in native systems; others may be more indirectly linked or associate in a rather dynamic way. One of these proteins, the β_2-adrenergic receptor (β_2AR), has been suggested as linker molecule coupling BK_{Ca} channels to Cav1.2 and to protein kinase A (PKA) and A kinase anchoring protein (AKAP150) (27, 39, 114). However, β_2AR is neither required for functional nor for biochemical coupling of BK_{Ca} and Cav1.2 channels as detailed above (12). Thus the biochemical evidence presented by Liu et al. (114) may point to a different, potentially more dynamic, role of β_2AR, as also suggested recently (38). Likewise, β-adrenergic receptors are known to trigger relaxation of smooth muscle cells via stimulation of BK_{Ca} channel activity through a signaling cascade involving activation of PKA (100). Whether this well-established mechanism requires direct interaction of BK_{Ca} channels with β_2AR, PKA/AKAP150 as suggested (55, 114) remains presently unclear; experiments in intact cells including colocalization by confocal immunofluorescence microscopy and topic β_2AR stimulation of BK_{Ca} currents in cell-attached patches (114) do not provide sufficient spatial resolution to selectively monitor coupling within protein complexes.

Another example is association of heme oxygenase 2 (HO-2) with BKα suggested to confer oxygen sensitivity to the channel (231). This complex has been proposed as a general oxygen sensor transducing moderate decreases in arterial partial pressure of oxygen into an afferent signal (93). Although direct interaction of BKα and HO-2 has been demonstrated in recombinant systems (231), existence and function of BK_{Ca}-HO-2 complexes in vivo is not yet clear. Thus hypoxia as well as carbon monoxide, a product of HO-2, have been shown to modulate native BK_{Ca} channels by different mechanisms that may not require a direct BK_{Ca}-HO-2 interaction (29, 93, 102, 119). Moreover, oxygen sensing was not affected in mice with targeted deletions of either HO-2 or BKα (152, 178).

These examples together with the large number of proposed but not thoroughly analyzed BK_{Ca} complexes (38, 120) seem to call for a more cautious use of the term *protein complex*. In any case, they emphasize the need for more detailed studies using advanced biochemical and functional techniques that are suited to properly characterize protein-protein interactions at resolution and stringency as outlined above.

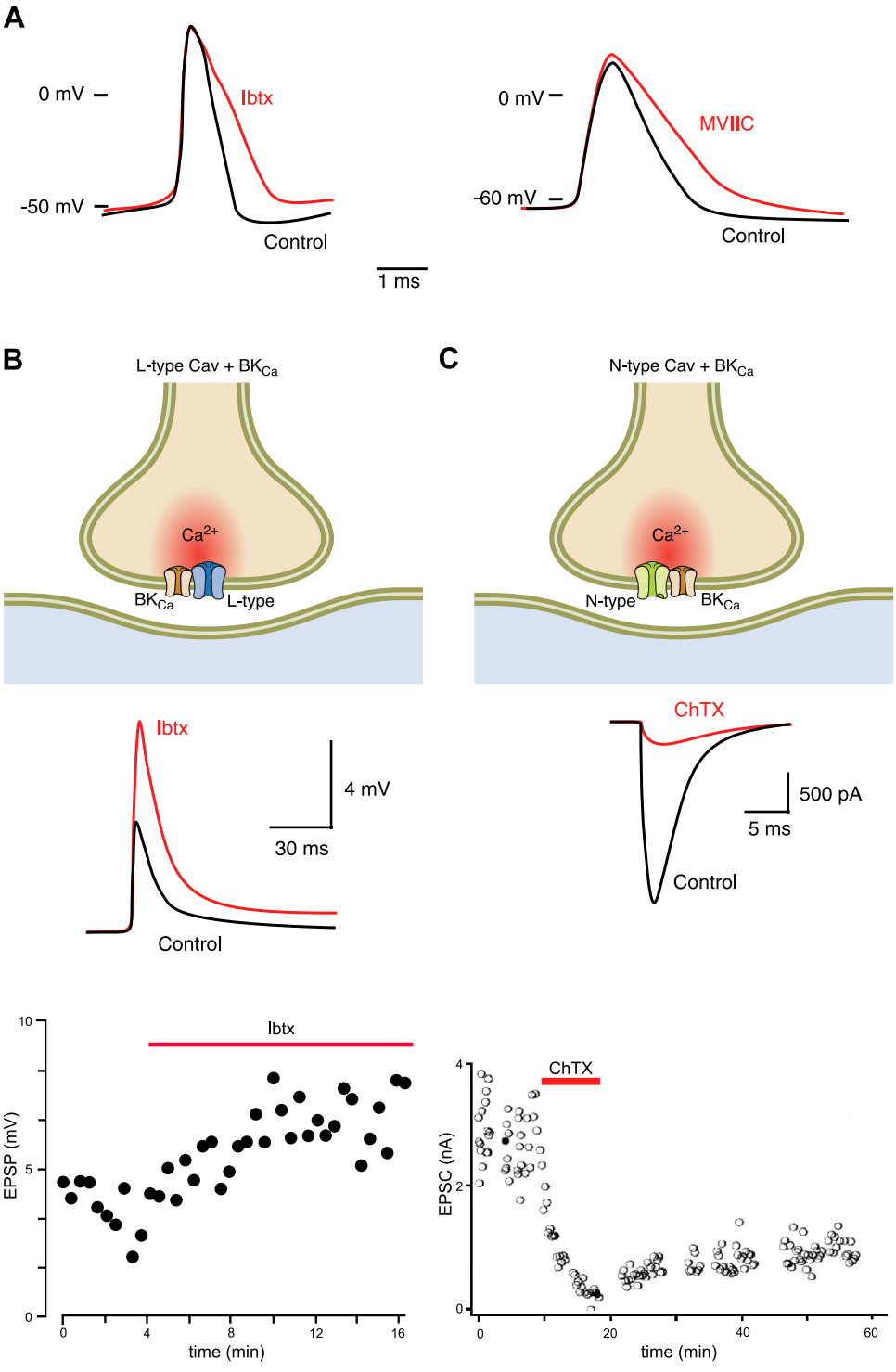


FIG. 4. Significance of BK_{Ca}-Cav complexes for AP repolarization and synaptic transmission. *A*: sharpening of APs by BK_{Ca}-Cav complexes visible upon block of either BK_{Ca} channels with iberiotoxin (Ibtx) [*left*; modified from Shao et al. (191)] or Cav channels with the peptide toxin MVIIC (blocker of Cav2.1/Cav2.2) [*right*; modified from Goldberg and Wilson (65)]. *B* and *C*: differential impact of distinct BK_{Ca}-Cav complexes on excitatory synaptic transmission. *B*: BK_{Ca} channels fueled by L-type Cav channels reduce amplitude and duration of EPSPs. [Modified from Grimes et al. (68).] *C*: BK_{Ca} channels activated by N-type Cav channels enhance the EPSC amplitude. [Modified from Pattillo et al. (156).]

III. MOLECULAR PARTNERS OF SK_{Ca} CHANNELS

The pore-forming α-subunits of SK_{Ca} channels (SKα) are encoded by four homologous genes, SK1–4 (or KCNN1–4) (83, 99) that are differentially expressed in neuronal and nonneuronal tissues (17, 121, 158, 195). While SK1 and SK2 are predominantly found in CNS neurons (SK2 also in sensory cells, microglia, urinary bladder, and cardiac myocytes), SK3 is expressed both in neuronal and glial cells as well as in diverse endothelial and smooth muscle cells (195). SK4 (also termed intermediate-conductance K^+ channel, IK1) is restricted to nonneuronal tissues such as muscle, epithelia, and blood cells (195).

SK2 comes in two variants probably resulting from different promotor usage, SK2-S (49 kDa) and SK2-L (78 kDa) (199); in addition, there are splice variants of SK1 (192), SK2 (137) and SK3 (213, 233), and the functional significance of this molecular diversity remains unclear. SKα, although sharing the tetrameric six-transmembrane domain architecture of voltage-gated cation channels, lacks the typical features of voltage-sensing S4 segments (Fig. 5*A*). Consequently, the gating of SK_{Ca} channels is fully independent of the transmembrane voltage (even at extreme membrane potentials), in contrast to BK_{Ca} channels. Instead, opening and closing of SK_{Ca} channels is solely driven by changes in $[Ca^{2+}]_i$, with submicromolar concentrations being sufficient to effectively gate the channels (76, 99).

A. Calmodulin, the β-Subunit of SK_{Ca}

Invariance of the channels' high sensitivity to Ca^{2+} in different experimental settings and cell types initially pointed towards a binding site located within the SKα protein. Finally, however, calmodulin (CaM) was identified as an exogenous Ca^{2+} sensor that is constitutively associated with SKα (242). SKα-CaM complexes are highly stable, form spontaneously in vitro (13), and have important structural, pharmacological, and functional implications. Although a number of other structurally related Ca^{2+}-binding proteins of the EF-hand protein family are expressed in brain, proteomic studies have underlined the exclusive role of CaM as the Ca^{2+} sensor of native SK_{Ca} channels (13).

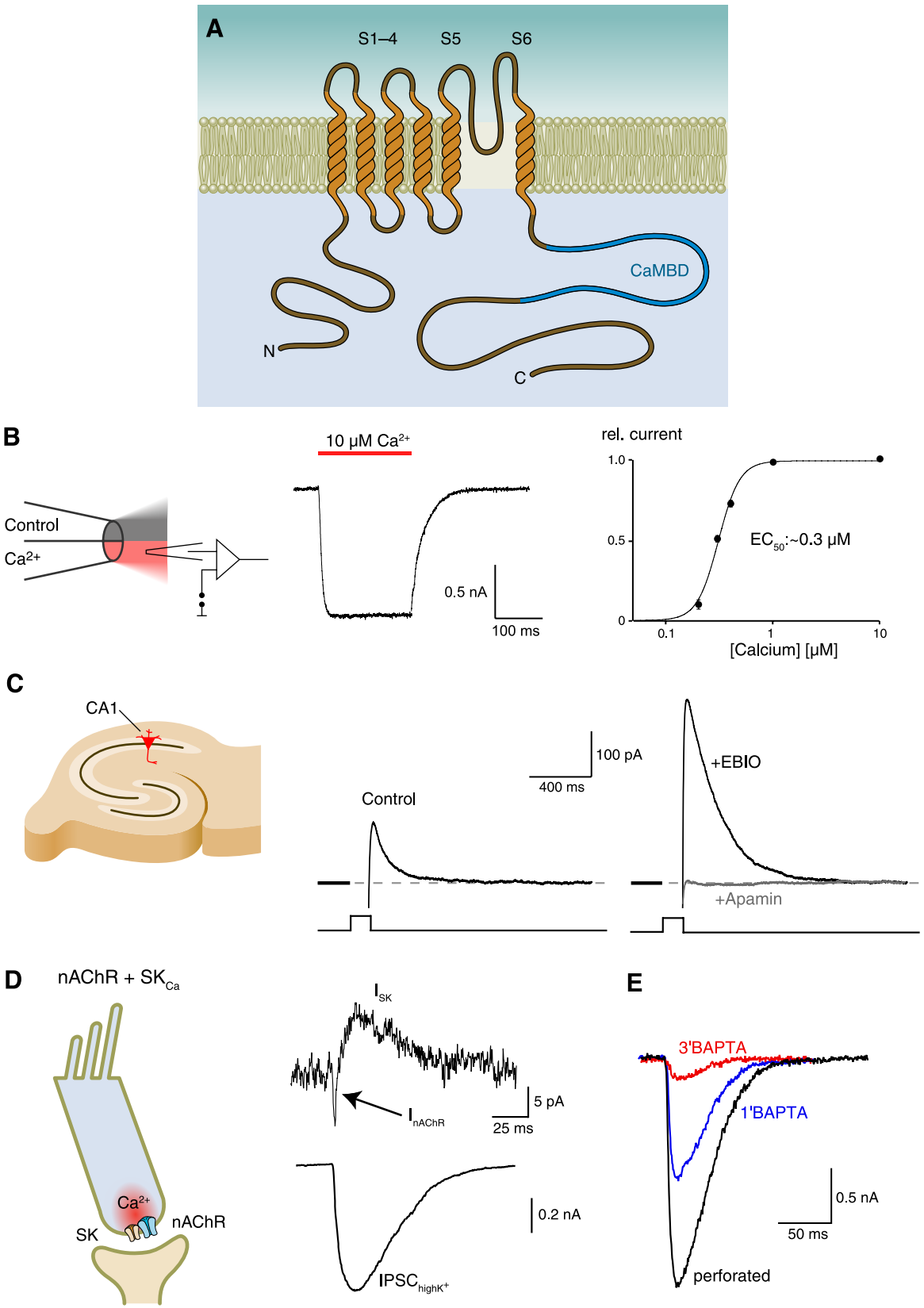
1. Biochemistry and structure

CaM is a small acidic (pI ~4.1) protein that is highly conserved among species and is ubiquitously expressed in eukaryotic cells. Basically, this cytoplasmic protein consists of a central region linking the two globular domains formed by the NH_2- and COOH-terminal half of the protein; each of the globular domains contains two EF hand-type motifs that bind Ca^{2+} with 1–3 μM (COOH-terminal lobe) and 10–20 μM (NH_2-terminal lobe) affinity in a positive-cooperative manner (134, 146, 159). Upon Ca^{2+} binding, the globular domains rearrange, bending the central connecting helix to form a hydrophobic clamp that interacts with target peptides of numerous effector proteins [reviewed by Chin and Means (30) and Rhoads and Friedberg (174)]. Binding of CaM to protein targets strongly increases its apparent Ca^{2+} affinity to low micromolar concentrations (134, 159). Different from this classical mechanism, the interaction of CaM with SKα displays two interesting facets. First, CaM constitutively interacts with SKα in the absence of Ca^{2+}; the respective interaction interface is made up from the NH_2- and COOH-terminal lobes of CaM and the proximal COOH terminus of SKα (so-called CaM binding domain, CaMBD; Fig. 5*A*; Refs. 92, 111, 188). Second, binding of Ca^{2+} only occurs at the NH_2-terminal EF-hands of CaM, which subsequently induces conformational changes and interactions with SKα that ultimately trigger opening of the channel pore by moving a gate located close to the intracellular part of the selectivity filter (23, 106, 242). The intimate association of SKα and CaM exhibits some distinct structural features as revealed by crystallography (187, 188) and NMR (232). The constitutive binding of CaM predominantly involves an extended helical stretch in the CaMBD representing a noncanonical CaM binding motif. Moreover, assembly with the CaMBD induces an unusual conformation that no longer allows for binding of Ca^{2+} to the COOH-terminal EF-hands (92, 188, 242). The structural rearrangements of SKα leading to opening of the pore have not yet been resolved; a dimer-symmetric transition has been proposed (187, 188), in line with the observed high positive cooperativity of Ca^{2+}-dependent gating (Hill coefficient ~4; see also sect. III*A2*).

Interestingly, mutagenesis experiments suggested that constitutive association with CaM is not an absolute requirement for Ca^{2+}-dependent opening of the channel pore, but necessary for cell surface trafficking of SK_{Ca} channels (88, 106). Accordingly, constitutive association with CaM may represent a mechanism ensuring that SK_{Ca} channels in the plasma membrane are saturated with its Ca^{2+} sensor in a 1:1 stoichiometry and are, therefore, able to respond to changes in $[Ca^{2+}]_i$ with high efficiency. Taken together, CaM is an auxiliary subunit required for proper channel function and may be regarded as the β-subunit of SK_{Ca} channels.

2. Modulation of channel gating

The functional properties of SK_{Ca} channels observed in heterologous expression systems largely parallel the behavior of their native counterparts. All four types of SK_{Ca} channels (when assembled with CaM) display a steep Ca^{2+} concentration-response relation with Hill coefficients of ~4 (~2.5 for SK4) and values for half-maximal activation (EC_{50}) of ~0.3 μM (Fig. 5*B*) (76, 99, 242). These values differ significantly from the low micromolar affinity and low positive cooperativity of most other Ca^{2+}-CaM triggered processes (134, 159) or the Ca^{2+} binding properties of free CaM (146, 159), emphasizing the impact of the SKα-CaM complexes. Experiments with coexpressed CaM mutants revealed the role of the individual Ca^{2+} binding sites for channel gating. Point mutations selectively reducing Ca^{2+} affinity in either of the two NH_2-terminal EF-hands (92) resulted in a reduction of both the apparent affinity as well as the steepness of the Ca^{2+} concentration-response relation. Accordingly, the large Hill coefficients appear to result from cooperative Ca^{2+} binding to the two NH_2-terminal EF-hands of CaM and a coupled conformational transition involving all four SKα subunits.



The opening of SK_{Ca} channels is the result of a coordinated sequence of events: Ca^{2+} binding to CaM and subsequent conformational changes within CaM and SKα that finally lead to structural rearrangements of the pore-occluding gate domain. The time constants of this activation process are strongly dependent on $[Ca^{2+}]_i$, but average to values of ~5 ms under saturating conditions (10 μM; Fig. 5*B*). Channel deactivation, the reverse process of activation initiated upon withdrawal of Ca^{2+}, is independent of $[Ca^{2+}]_i$ and occurs on a considerably slower time scale with time constants ranging from 15 to 60 ms (Fig. 5*B*, see also sect. III*B2*) (76, 157, 242). These gating properties endow SK_{Ca} channels with a "short-term memory" for $[Ca^{2+}]_i$, i.e., they remain active for more than 100 ms after $[Ca^{2+}]_i$ has returned to resting levels and are able to integrate even low-frequency Ca^{2+} signals over time.

The deactivation process of SK_{Ca} channels can be slowed ~10-fold by the small synthetic molecule 1-ethyl-2-benzimidazolinone (1-EBIO) (40) which at millimolar concentrations effectively stabilizes the open state of the channels (157). As a consequence, the dose-response relation is shifted into the lower nanomolar range promoting robust activation of SK_{Ca} channels even at resting levels of $[Ca^{2+}]_i$ (<100 nM) (148, 157). Meanwhile, a number of different compounds that positively (e.g., NS309) (201) or negatively (e.g., NS8593) (200) modulate SK_{Ca} channels have been described [reviewed by Pedarzani and Stocker (158)]. It should be noted that these compounds specifically act on SKα-CaM complexes (111, 200, 201); effects on other CaM-dependent processes have not been described so far. Conversely, classical CaM antagonists failed to inhibit activation of SKα-CaM (242), underlining the importance of protein complexes as structural determinants for the pharmacology of proteins and as targets for the development of novel drugs (14).

3. Implications for cell physiology

The properties of the SKα-CaM based Ca^{2+} gating machinery are fundamental for the role of SK_{Ca} channels in the cellular context, where these channels reconstitute an interactive feedback between excitability and $[Ca^{2+}]_i$. In fact, this feedback may be distinct in different types of cell, but essentially falls in between two extremes.

First, SK_{Ca} channels may integrate multiple Ca^{2+} signals over time to modulate repetitive electrical activity and firing pattern. An example for this type of feedback is the medium phase afterhyperpolarization (mAHP) that may last from a few tens to a few hundreds of milliseconds (Fig. 5*C*) (16, 196). This mAHP was observed following depolarizing pulses and APs in several types of central neurons (81, 117, 235) where it promoted successive slowing of the spike frequency (157, 196). Moreover, this feedback mechanism controls pacemaking (234) and contributes to integration of synaptic input in dendrites (24). Application of 1-EBIO was found to largely enhance these SK_{Ca}-mediated effects (Fig. 5*C*) (72, 157), indicating that the limiting factor for the underlying SK_{Ca} channel activity is the spatial and kinetic extension of $[Ca^{2+}]_i$ profiles sufficiently high to open SK_{Ca} channels.

The second type of feedback refers to SK_{Ca} channels shaping single Ca^{2+} events, mainly by the slow deactivation kinetics. Thus SK_{Ca} channels in postsynaptic compartments provide hyperpolarizing K^+ currents that limit the Ca^{2+} influx through NMDA-type glutamate receptors by promoting their pore-block via Mg^{2+}; as a consequence, SK_{Ca} channels were found to shape the excitatory postsynaptic currents (EPSCs) (49, 143) and affect the induction of synaptic plasticity (73, 112). In auditory hair cells, SK2 channels driven by Ca^{2+}-permeable nicotinic acetylcholine receptors of the $\alpha9/\alpha10$ subtype give rise to an inhibitory postsynaptic current (IPSC) carried by K^+ (148, 246). The time course of this IPSC is determined by the gating kinetics of the SK_{Ca} channels (Fig. 5*D*) (148). In nonneuronal cells, SK_{Ca} channels control the Ca^{2+} influx through Ca^{2+} sources usually gated on a slower time scale than in neurons. Thus, in smooth muscle cells, SK_{Ca} channels regulate contractility by limiting Ca^{2+} influx without exerting a prominent effect on the shape of the slow APs promoting the Ca^{2+} influx and activation of the SK_{Ca} channels (75). Another example is offered by the role of SK4 channels for the activation of T lymphocytes (reviewed in Ref. 154). Stimulation of T-cell receptors initiates Ca^{2+} release from intracellular stores, which subsequently activates Ca^{2+} release-activated Ca^{2+} channels and SK4 channels; both types of channels remain tonically active with the SK4-mediated K^+ current maintaining the driving force required for sustained Ca^{2+} influx (51).

FIG. 5. Properties of recombinant and native SK_{Ca} channels. *A*: membrane topology of SK_{Ca} α-subunits as described in the text. *B*: Ca^{2+}-dependent gating of heterologously expressed SK2 channels recorded in excised inside-out patches upon rapid application of 10 μM Ca^{2+} (*left* and *middle*). *Right*: Ca^{2+} concentration-response relation of SK2 channels (242). *C*: SK_{Ca}-mediated currents recorded in hippocampal CA1 neurons in response to a depolarizing pulse (10 mV, 100 ms) under control conditions (*middle*) and after incubation with 2 mM 1-EBIO (*right*). [Adapted from Pedarzani et al. (157).] *D*: SK_{Ca}-mediated IPSCs in auditory outer hair cells (OHCs) of the rat organ of Corti (*left*). *Middle*: spontaneously evoked IPSCs at physiological (*top trace*) and symmetrical high K^+ concentrations (*bottom trace*). Note the biphasic response observed with physiological K^+: a brief inward current through nicotinic acetylcholine receptors (I_{nAchR}) followed by a K^+ outward current mediated by SK2 channels (I_{SK}). *Right*: overlaid IPSCs measured before (black), after 1 min (blue), and after 3 min (red) of 5 mM BAPTA dialyzed into the OHC. [Recordings from Oliver et al. (148).]

The exquisitely high affinity for Ca^{2+} should relieve SK_{Ca} channels from the requirement of a close interaction with Ca^{2+} sources, and rather allow for their effective activation even if located at distances of up to a few hundred nanometers remote from such a source (50). However, a number of studies reported defined localization of SK_{Ca} channels relative to specific Ca^{2+} sources. Thus distances of between some 10 and ~150 nm have been derived for SK2 and either L-type Cav channels in the soma of CA1 pyramidal cells (126), or NMDA receptors in hippocampal CA1 neurons (143), or $\alpha9/\alpha10$ acetylcholine receptors in auditory outer hair cells where high concentrations of EGTA were ineffective in preventing activation of SK2 channels (Fig. 5*E*) (148). Another hint for defined localization comes from activation of SK_{Ca} channels by distinct Ca^{2+} sources over others. Thus SK_{Ca} channels appear to be selectively activated by L-type Cav channels in the somata of CA1 pyramidal cells (126), by R-type Cav channels in dendritic spines of CA1 and lateral amygdala pyramidal cells (15, 49), or by T-type Cav channels and SERCAs in neurons of the thalamic nucleus reticularis (36). The mechanisms underlying such specificity remain unclear; so far, neither direct nor indirect association of SK_{Ca} channels with Ca^{2+} sources has been substantiated by biochemistry.

It must be emphasized, however, that activation of SK_{Ca} channels at a given distance from any Ca^{2+} source is strongly dependent on the conductance properties of the source. With this respect, Cav channels and the Ca^{2+}-permeable NMDA and $\alpha9/\alpha10$ acetylcholine receptors exhibit conductances for Ca^{2+} that are 5- to 10-fold different from each other (Cav channels, <10 pS; NMDA receptors, >50 pS; $\alpha9/\alpha10$ receptors, 70 pS) (32, 98, 165, 230). Accordingly, the ionotropic receptors will activate SK_{Ca} channels at larger distances and more effectively than Cav channels.

B. Complexes of SK_{Ca} Channels

1. *Biochemistry and structure of SK_{Ca} channel complexes*

Heterogeneity observed with the kinetics of SK_{Ca}-mediated currents in various types of neurons together with strong effects observed with allosteric activators and inhibitors [reviewed in Pedarzani and Stocker (158)] suggest that the gating machinery may be a target for physiological regulation and prompted efforts to identify associated regulatory proteins. Bildl et al. (13) using a proteomic approach identified subunits of protein kinase CK2 and protein phosphatase 2A (CK2 catalytic α- and regulatory β-subunit, PP2A regulatory subunits $PP2A_A$/PR65 and $PP2A_B$/PR55) as specific interactors at the COOH termini of SK2 and SK3 (13). In vitro binding tests and yeast two-hybrid assays confirmed that these proteins together with CaM and SK2/3 NH_2 and COOH termini coassemble into a polyprotein complex (Fig. 6). The resulting compact structure is stabilized through a network of interactions between distinct domains of individual subunits, for which electrostatics play a prominent role (3, 92, 188). More explicitly, CaM, CK2α, CK2β, and $PP2A_A$ coassemble both with the CaMBD and the SK2/3 NH_2 terminus, thus bridging the intracellular domains of SK_{Ca} channels. The catalytic subunit of protein phosphatase 2A ($PP2A_C$) may interact with the SK COOH terminus as well as with $PP2A_A$ and CK2α (Fig. 6) (13, 33). Stable integration of CK2 into native SK-CaM complexes has been confirmed by affinity copurification (13). In contrast, PP2A was not detected in native SK_{Ca} channels, indicating that its association may be regulated or less stable in vivo.

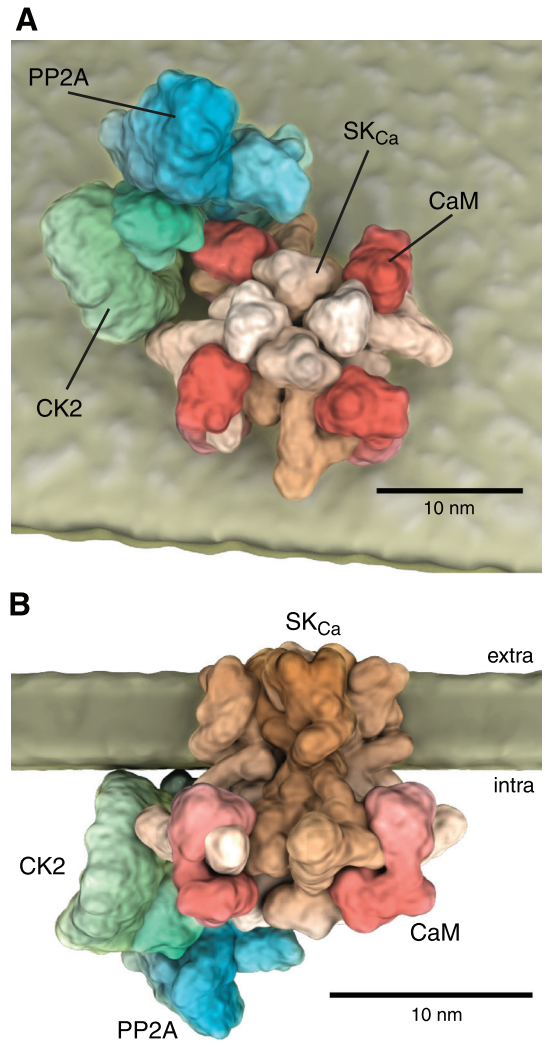


FIG. 6. Structural model of a SK_{Ca} channel complex composed of SKα, CaM, CK2, and PP2A. Top view (from cytoplasmic side, *A*) and side view (*B*) of a space-filling model of a SK_{Ca} supercomplex assembled from SKα (brown), CaM (red), CK2 (green), and PP2A (blue) generated as in Fig. 2 using database entries for SK_{Ca}/CaM (2PNV, 1KKD, 1G4Y, 1QX7), Kv1.2 (2A79), CK2 (1JWH), and PP2A (2IAE); scale bar is 10 nm.

2. Functional analysis of synthetic SK_{Ca} channel complexes

Protein kinase CK2 preferentially phosphorylates serine and threonine residues in the context of acidic residues and requires clustered positive charges for its activation (144). CaM, a well-known substrate for CK2 in its apo form (131, 140), is effectively phosphorylated by CK2 when offered in complex with the CaMBD that harbors several stretches of basic residues (13). Further analysis using site-directed mutants finally identified threonine-80 in CaM as the target for CK2-mediated phosphorylation and as a major determinant for gating of SK_{Ca} channels. When dephosphorylated at this residue, as mimicked by an alanine substitution [CaM(T80A)], the SKα-CaM complex exhibited a Ca^{2+} sensitivity in the submicromolar range. Upon phosphorylation [aspartate substitution, CaM(T80D)], however, the Ca^{2+} sensitivity shifted into the micromolar range, and the steepness of the Ca^{2+} concentration-response relation was markedly reduced (Fig. 7*B*); in addition, the CaM(T80) mutants abolished the effects of CK2 and ATP on channel gating (13). The reduction in apparent Ca^{2+} affinity resulted from accelerated channel deactivation, likely reflecting increased dissociation rates for Ca^{2+} from their CaM binding site(s). The respective deactivation time constants exhibited values of ~60 and ~14 ms for the dephospho and phospho state of CaM, respectively (13). In the context of the SK_{Ca} channel complex, the phosphorylation status of threonine-80 may either be balanced by the activities of CK2 and PP2A or shifted towards the dephospho or phospho state by regulation of the respective enzymatic activity. In summary, the integration of CaM, CK2, and PP2A into a stable complex provides a potentially rapid and potent switch for bidirectional modulation of SK_{Ca} channel activity.

3. Implications for cell physiology

Modulation of SK_{Ca} channels through phosphorylation of CaM may have different physiological implications, depending on the Ca^{2+} signal. Under conditions of limiting $[Ca^{2+}]_i$, it should decrease the steady-state activity and accelerate the decay of SK_{Ca} currents. In addition, this modification should largely impair the channels' ability to act as integrators of rapid Ca^{2+} signals due to both slowing of activation (157) and acceleration of deactivation (13). Under saturating Ca^{2+} conditions, phosphoregulation of the deactivation kinetics should effectively alter the decay of postsynaptic currents and the duration of AP-induced afterhyperpolarization.

So far, the physiological significance of these mechanisms has been supported by two examples: catecholamine-mediated sensitization of sensory neurons and efferent inhibition of cochlear hair cells. Nociceptive neurons in the dorsal root ganglion (DRG) are sensitized by the neurotransmitter noradrenaline (NE; reviewed by Pertovaara, Ref. 161) through a signaling cascade involving inhibition of SK_{Ca} channels (6, 124). As the underlying molecular mechanism, NE was shown to activate CK2, which through subsequent phosphorylation of SKα-bound CaM reduced the amplitude of AP-triggered SK_{Ca} currents (124). Some key elements of this cascade are depicted in Figure 7. Functional coupling of single SK_{Ca} and Cav channels in membranes of superior cervical ganglion (SCG) neurons was largely abolished after application of NE (Fig. 7*C*). The underlying CK2-mediated phosphorylation of CaM manifested as a reduced Ca^{2+} affinity of the SK_{Ca} channels, comparable to the phosphorylation-mimicking CaM(T80D) mutation. DRG neurons, which under control conditions showed effective SK2-mediated spike-frequency adaptation (Fig. 7*D*, *left*), fired multiple APs upon NE-triggered inhibition of SK_{Ca} channel activity (Fig. 7*D*, *right*) (124).

SK2-IPSCs in outer hair cells (OHCs) and immature inner hair cells (IHCs) vary with respect to their decay time constants from 18–55 ms, which actually covers the full range set forth by the CaM(T80D) and CaM(T80A) mutants (13, 148). Stimulation of CK2 activity induces and maintains rapid IPSC deactivation kinetics, indicating that the machinery for modulation of the Ca^{2+} sensitivity is present in these cells. At present, this regulatory mechanism is implied in suppression of Ca^{2+} APs in immature IHCs prior to the onset of hearing (67), and in hyperpolarization shunting of OHCs to limit the amplitude of the receptor potential and the active cochlear amplification during periods of high sound-pressure levels (125, 138).

IV. CONCLUSIONS AND FUTURE DIRECTIONS

The key function of BK_{Ca} and SK_{Ca} channels in vertebrates to provide membrane hyperpolarization in response to elevation of $[Ca^{2+}]_i$ is critically dependent on their integration into complexes with other proteins. BK_{Ca} channels requiring $[Ca^{2+}]_i$ in the micromolar range for reliable activation under physiological conditions form stable macromolecular complexes with a specific set of Cav channels (Cav1.2, Cav2.1, and Cav2.2) and a set of BKβ proteins that fine-tune the channels' gating machinery with respect to Ca^{2+} and voltage dependence. SK_{Ca} channels form constitutive complexes with CaM endowing them with a gating apparatus that responds to submicromolar $[Ca^{2+}]_i$ with high cooperativity; sensitivity and dynamic range of this apparatus are modulated through phosphorylation/dephosphorylation of CaM via coassembled CK2 and PP2A. In either case, the stable association with partner proteins is used to optimally adapt the channels to their distinct and cell type-specific physiological functions.

In this review, we focused on what may be regarded the "inner core" of K_{Ca} channels formed by protein-protein in-

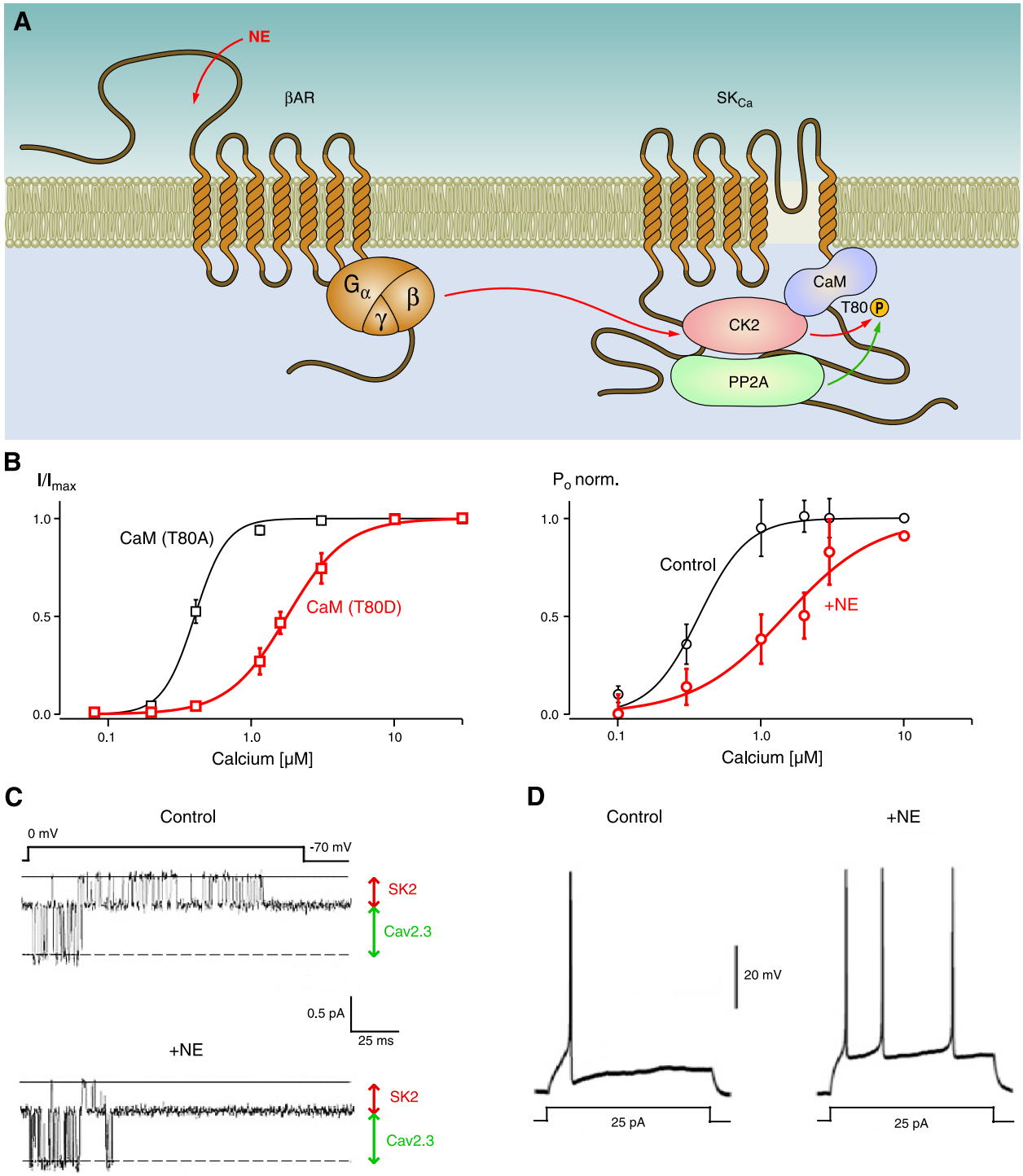


FIG. 7. Ca^{2+} sensitivity of SK_{Ca} channels is shifted by CK2-mediated phosphorylation of CaM. *A*: signaling cascade underlying modulation of SK_{Ca} channels by β-adrenergic receptors (βAR). *B*: Ca^{2+} concentration-response relation of heterologously expressed SK2 channels assembled with the indicated phospho-mimicking mutants (*left*) and of SK_{Ca}-mediated currents recorded in SCG neurons before and after stimulation with norepinephrine (NE) [*right*; modified from Maingret et al. (124)]. *C*: activation of SK_{Ca} channels by Cav2.3 (*top trace*, control) is largely suppressed upon application of NE (*bottom trace*). *D*: AP responses to current injections in DRG neurons displaying SK_{Ca}-mediated spike-frequency adaptation (*left*) that is largely reduced by NE. [Modified from Maingret et al. (124).]

teractions with high affinity and limited association/dissociation dynamics. However, there are a number of aspects and questions regarding these inner cores that may be of a more general relevance assuming that the principles derived from K_{Ca}-associated complexes also apply to complexes assembled with other membrane proteins (38, 107, 186).

Although apparently stable at the plasma membrane, the dynamics and life cycle of K_{Ca}-associated complexes in the cellular environment are largely unresolved. In particular, the factors driving assembly, subcellular targeting, and biological turnover have not yet been elucidated, and it is unknown whether "stable" protein complexes undergo remodeling (exchange of subunits) in vivo.

The allosteric (reciprocal) nature of protein-protein interactions suggests that coassembly may lead to structural and functional changes affecting all partners as evident for the association of SKα with CaM and CK2. In this case, coassembly alters both the Ca^{2+} binding properties of CaM and the catalytic activity of the kinase.

A hallmark of protein complexes is the multifunctionality of their subunits. Proteins partnering with K_{Ca} influence, at the same time, gating, pharmacology, and cell surface expression (and potentially other properties including biological half-life or targeting to specific compartments). Moreover, some of these proteins are known to also act as subunits of other protein complexes where they serve related or different functions. This molecular organization generates a high functional and cell type specific diversity using a limited set of genes.

Current biochemical methods may miss more transient or low-affinity interaction partners such as kinases or signaling mediators that may, nevertheless, be functionally important. This would explain some of the observed mismatches between biochemistry and functional data. It should also be kept in mind that not all biochemical interactions may have direct functional implications and, conversely, that even tight functional coupling of proteins does not per se require a direct molecular interaction.

The inner core most likely represents only one layer of the higher order molecular organization in cells. Thus BK_{Ca} and SK_{Ca} channels may interact with other protein complexes to form supercomplexes or integrate into extended protein networks. Analysis of these structures represents as yet unmet biochemical challenges in terms of solubilization, specific isolation, and heterogeneity/complexity of their composition.

Regarding the significant number of candidate partners proposed for K_{Ca} channels, compelling evidence for integration into complexes has so far only been obtained for a limited set of proteins. In our view, the definition of protein complexes as well as the issues listed above would strongly benefit from more advanced experimental strategies and technologies. Adequately controlled biochemistry (complex solubilization and purification) coupled to unbiased and quantitative analysis by mass spectrometry can strongly minimize errors and provide additional information on direct versus indirect interactions and their stability (63, 185). Likewise, novel high-resolution imaging techniques and structural methods will allow direct characterization of protein complexes in native systems. In the light of these recent technical advancements, more exciting discoveries on protein complexes are expected to come.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

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