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SYMPOSIUM REVIEW

Mechanisms and dynamics of AKAP79/150-orchestrated multi-protein signalling complexes in brain and peripheral nerve

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Abstract A-kinase anchoring proteins (AKAPs) have emerged as a converging point of diverse signals to achieve spatiotemporal resolution of directed cellular regulation. With the extensive studies of AKAP79/150 in regulation of ion channel activity, the major questions to be posed centre on the mechanism and functional role of synergistic regulation of ion channels by such signalling proteins. In this review, we summarize recent discoveries of AKAP79/150-mediated modulation of voltage-gated neuronal M-type (KCNQ, Kv7) K^+ channels and L-type Ca_V1 Ca^{2+} channels, on both short- and longer-term time scales, highlighting the dynamics of the macromolecular signalling complexes in brain and peripheral nerve We also discuss several models for the possible mechanisms of these multi-protein assemblies and how they serve the agenda of the neurons in which they occur.

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Abbreviations AKAP, A-kinase anchoring protein; CaN, calcineurin; CaM, calmodulin; DAG, diacylglycerol; DN, dominant negative; IP_3, inositol trisphosphate; L-channels, L-type Ca^{2+} channels; M-channels, KCNQ (M-type) K^+ channels; NFAT, nuclear factor of activated T-cells; PIP_2, phosphatidylinositol 4,5-bisphosphate; PKA, protein kinase A; PKC, protein kinase C; STORM, stochastic optical reconstruction microscopy; WT, wild type.

Introduction

A-kinase anchoring proteins (AKAPs) are a diverse scaffold protein family that orchestrates enzymes with their substrates into protein complexes, which ensures specificity and optimal facilitation of signal transduction (Bauman *et al.* 2006). Over 70 AKAPs have been identified in different cell types. They all share

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the common ability to bind protein kinase A (PKA), as well as roles in organizing multi-protein signalling complexes in specific subcellular locations (Wong & Scott, 2004). The well-studied isoforms AKAP79/150 (human AKAP79/rodent AKAP150) are widely expressed in the nervous system, and regulate diverse neuronal ion channels through phosphorylation or dephosphorylation by signalling proteins such as PKA, PKC, calmodulin (CaM), calcineurin (CaN), and phosphatidylinositol 4,5-bisphosphate (PIP_2). The channels modulated include AMPA-type glutamate receptors, the inwardly rectifying potassium channel Kir 2.1, L-type Ca^{2+} channels (L-channels), KCNQ (M-type) K^+ channels, TRPV cation channels, and various G protein-coupled receptors, making it a point of convergence for integration of diverse signals, as discussed in previous reviews (Wong & Scott, 2004; McConnachie *et al.* 2006; Esseltine & Scott, 2013). In this mini-review, we focus on the novel discoveries of AKAP79/150 in directing multi-protein signalling complexes with reference to M-type (K_v7) K^+ channels and L-type (Ca_V1) Ca^{2+} channels, and the mechanisms and dynamics of these complexes in the nervous system.

AKAP79/150 actions on M-type K^+ channels: altered PIP_2 sensitivity, and effects on CaM interactions

Voltage-gated M-type (KCNQ, Kv7) K^+ channels, expressed in a wide variety of neurons, play critical roles in modulation of excitability and action potential firing (Brown & Adams, 1980; Constanti & Brown, 1981; Delmas & Brown, 2005; Hernandez *et al.* 2008). Heteromers of KCNQ2 and KCNQ3, which were identified from an inherited human neonatal form of epilepsy called benign familial neonatal convulsions, underlie neuronal M-current. Given their critical role in regulation of neuronal excitability, M-channels are closely regulated by various neurotransmitters through three mechanisms from four related second messenger signalling events in neurons: First, M-channels are highly sensitive to the binding of membrane PIP_2; thus, depletion of membrane [PIP_2] or change in the PIP_2 sensitivity of the channels strongly depress M-current by reducing the open probability of the channel (Suh & Hille, 2008; Logothetis *et al.* 2010). Such unbinding of PIP_2 comes from stimulation of $G_{q/11}$-coupled neurotransmitter receptors that activate phospholipase C (PLC), which hydrolyses PIP_2 to diacylglycerol (DAG) and inositol trisphosphate (IP_3). Secondly, DAG and IP_3 generated by PIP_2 breakdown potentially activate PKC and generate rises in intracellular Ca^{2+} (Ca_i^{2+}) from internal Ca^{2+} stores. Third, Ca^{2+}–CaM binds to and suppresses M-channels (Gamper & Shapiro, 2003; Delmas & Brown, 2005). Fourth, AKAP79/150 physically associates with KCNQ2-5 subtypes, recruiting PKC and facilitating subsequent phosphorylation and enhancing suppression of KCNQ channels (Hoshi *et al.* 2003; Bal *et al.* 2010; Zhang *et al.* 2011). These related, but seemingly complex and redundant, signalling pathways underlying M-channel modulation made us inquire as to the physiological function of AKAP-PKC on M-channels, synergistic with PIP_2 reduction and CaM action.

One attractive model that synergizes PIP_2, Ca^{2+}, CaM and PKC actions involves allosteric effects of one signalling molecule on the affinities of the others for KCNQ channels, with the focus on AKAP79/150 (Kosenko *et al.* 2012; Kosenko & Hoshi, 2013; Fig. 1). For the case of PKC, association of apoCaM with the A and B helices of the C-terminus of the channels is obligatory to maintain tonic PIP_2 affinity (Kosenko *et al.* 2012). AKAP79/150-mediated PKC phosphorylation of KCNQ channels is suggested to induce rearrangement of CaM on the C-terminus of the channels, resulting in lowered PIP_2 affinity and sensitization to muscarinic depression of M-current. At the same time, Ca^{2+} binding to CaM is suggested to likewise induce a conformational change in CaM bound to the channels, again reducing the channel–PIP_2 affinity (Kosenko & Hoshi, 2013). Such a conformational change is consistent with the crystal structure of Ca^{2+}–CaM bound solely to the KCNQ channel B helix (Xu *et al.* 2013*b*), whereas apoCaM likely cross-links the A and B helices (Wen & Levitan, 2002; Yus-Najera *et al.* 2002; Gamper & Shapiro, 2003; Xu *et al.* 2013*b*). Interestingly, the Hoshi group has suggested that AKAP79/150 acts as an acceptor for CaM dissociated from the channels after their PKC phosphorylation or Ca_i^{2+} increases (Kosenko *et al.* 2012; Kosenko & Hoshi, 2013), a hypothesis that would seem at odds with maintained CaM binding to the channels regardless of Ca^{2+} binding (Bal *et al.* 2008).

We recently suggested a dual mode of mixed convergence–competition model to explain AKAP79/150 involvement in M-current suppression by only one group of neurotransmitter receptors that are localized away from ER Ca^{2+} stores and do not induce IP_3-mediated Ca_i^{2+} rises (Fig. 2*A*; Zhang *et al.* 2011). Our thinking is based on the following observations: (1) these receptors physically associate with AKAP79 (Hoshi *et al.* 2003; Bal *et al.* 2010), (2) AKAP79/150 and CaM share overlapping binding sites on the C-terminus of KCNQ channels, and Ca^{2+}–CaM disrupts AKAP79/150-channel interactions (Bal *et al.* 2010), and (3) over-expression of WT, but not DN, CaM prevents AKAP79/150-mediated channel sensitization (Bal *et al.* 2010). In this model, rearrangement of CaM induced by Ca^{2+} binding prevents AKAP79/150-mediated PKC phosphorylation by interfering with the association of AKAP79/150 with KCNQ complexes (Fig. 2*B*). Association of receptors with those complexes is required to co-localize M-channels with putative transient, local depletions of PIP_2 and activation of PKC that may be more physiologically relevant (Fig. 2*A*). Thus, M-channels reduce their

activity in response to two types of concurrently sensed responses: *either* PIP_2 depletion + AKAP79/150-mediated PKC phosphorylation (Fig. 2*A*), *or* IP_3-mediated Ca^{2+} release + Ca^{2+}–CaM actions on M-channels (Fig. 2*B*). Such convergent dual mechanisms may serve as a coincidence-detector to fine tune the spatiotemporal resolution of directed signals.

Crucial questions arising from the two models are (1) whether CaM constitutively binds to KCNQ channels in native neurons, (2) whether CaM–KCNQ association is obligatory for channel activity, and (3) whether expression of functional channels or Ca_i^{2+} -sensing is the more critical role of CaM. At this moment, it is hard to answer question (3), but biochemical studies pointing out both a

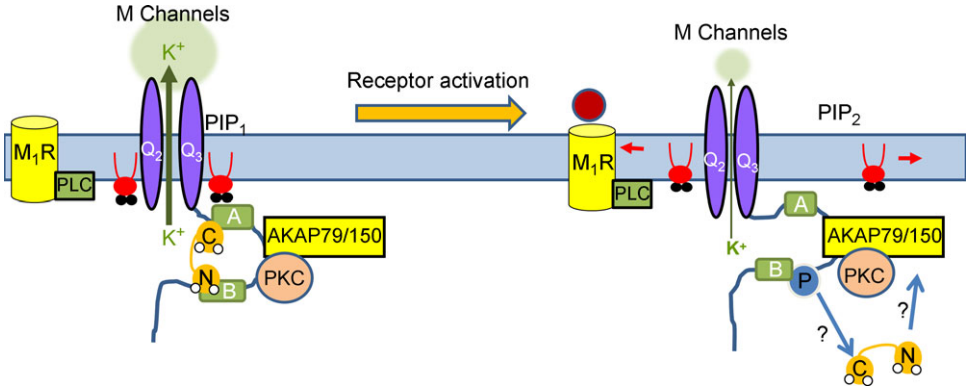

Figure 1. Model of 3-step rearrangement of AKAP–KCNQ channel complex
See Kosenko *et al*. (2012) and Kosenko & Hoshi (2013). AKAP79/150 organizes a signalling complex at the carboxyl terminus of KCNQ channels, including PKC and CaM. Stable KCNQ–CaM association is required for maintaining tonic PIP_2 affinity. Stimulation of $G_{q/11}$-coupled muscarinic receptors depletes PIP_2 and activates AKAP79/150-anchored PKC, which phosphorylates KCNQ subunits near the CaM binding sites of KCNQ channels. PKC phosphorylation, or Ca_i^{2+} signals, triggers CaM conformational changes and/or dissociation from the cytoplasmic tail of KCNQ channels, resulting in lowered PIP_2 affinity and suppressed channel activity. AKAP79/150 may act as an acceptor to bind to dissociated CaM. Thus, together with PIP_2 depletion, receptor stimulation suppresses KCNQ channel activity by inducing CaM rearrangement and dissociation from the channel complex. Note that the depression of M-current by reducing channel opening is schematically indicated here by a squeezing together of the two halves of the channel, and a thinning of the directional arrow of potassium flux. See the text for issues concerning this model.

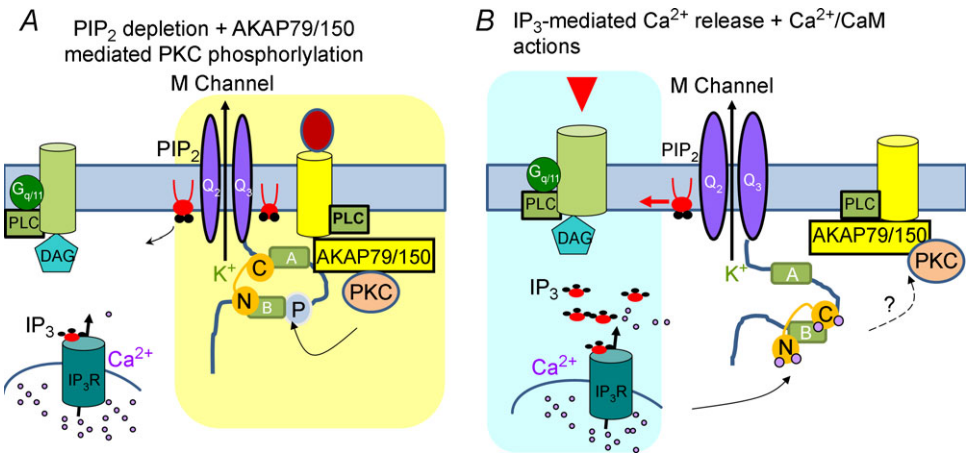

Figure 2. Modified concurrent dual mode of KCNQ channel inhibition
In this model, phosphorylation of KCNQ channels functions to reduce their PIP_2 affinity, priming them to small changes in PIP_2 abundance induced by physiological stimulation of receptors. *A*, activation of receptors that are part of AKAP79150–KCNQ complexes rapidly depletes PIP_2 and activates AKAP79/150-anchored PKC, which phosphorylates KCNQ subunits near the CaM binding site on the B helix, altering the configuration of CaM and KCNQ channels. KCNQ channels thus sense both PIP_2 depletion and phosphorylation-mediated PIP_2-affinity reduction. *B*, activation of receptors located close to IP_3 receptors, but outside of the AKAP–KCNQ complexes, induces intracellular Ca^{2+} rises and Ca^{2+} binding to CaM, which undergoes a conformational change on the overlapping binding sites with AKAP150 on the carboxy-terminus of KCNQ channels. This induces a lowered interaction between KCNQ and AKAP79/150, thus preventing PKC phosphorylation.

changed conformation and affinity of the CaM–KCNQ complex after Ca^{2+} binds (Black & Persechini, 2011; Wang *et al.* 2013; Alaimo *et al.* 2014) suggest it is likely that these two mechanisms are not exclusive. Recently, it has been shown that the N-lobe of apoCaM binds to helix B due to its greater affinity, whereas the C-lobe moves from helix A to helix B upon Ca^{2+} binding (Alaimo *et al.* 2014). Our current thinking has thus been modified accordingly (Fig. 2): (1) cross-linking of apoCaM with helices A and B is important to maintain their helical structure, which we suppose is critical to stabilize channel interactions with PIP_2; (2) PKC phosphorylation on helix B (Fig. 2*A*) weakens the interaction between helix B and the N-lobe of CaM, resulting in reduced PIP_2 affinity and suppressed M-current (consistent with both models), and (3) maximal Ca^{2+} binding to CaM (Fig. 2*B*) induces the C-lobe to move from helix A to helix B and a subsequent conformational change of both CaM and KCNQ channels which causes reduced PIP_2 affinity, weaker AKAP79/150 association with (and perhaps AKAP79/150 dissociation from) the channel complex and prevention of PKC phosphorylation (Fig. 2*B*). Further direct biochemical analysis in native neurons of KCNQ channels, AKAP79/150 and CaM would be very helpful, and direct observation of native KCNQ channel–AKAP150–CaM dynamics upon receptor stimulation would be exciting.

AKAP79/150 interactions on L-type Ca^{2+} channels: priming by PKA and Ca^{2+} sensing by CaN

Another ion channel family whose activity is under close regulation is voltage-gated Ca^{2+}channels (VGCCs), especially L-type (Ca_V1) channels which are critical for synaptic plasticity, axon growth and neuronal development. In the hippocampus, AKAP79/150 bound to the modified leucine zipper (LZ) motif of the distal C-terminus of $Ca_V1.2$ channels anchors PKA to phosphorylate and up-regulate channel activity, whereas the Ca^{2+}–CaM-regulated phosphatase, calcineurin (CaN), which is also recruited by AKAP79/150, counter-balances PKA actions, serving as an activity-dependent negative feedback mechanism (Hall *et al.* 2007; Oliveria *et al.* 2007). Moreover, AKAP79/150 intimately regulates gene transcription via Ca^{2+}–CaN, inducing CaN-mediated NFAT (nuclear factor of activated T-cells) dephosphorylation and translocation to the nucleus, where NFAT interacts with regulatory elements of NFAT-sensitive genes (Graef *et al.* 1999, 2003; Hudry *et al.* 2012; Wu *et al.* 2012; Murphy *et al.* 2014). We have recently identified similar L-channel–AKAP–CaN protein complexes in sympathetic ganglia that initiate NFAT signalling to up-regulate M-channel expression, serving as a longer-term feedback mechanism to counter increased neuronal hyperexcitability (Zhang & Shapiro, 2012). We suggest that upregulating M-channels is a critical 'anti-epileptogenic' mechanism in brain regions such as the hippocampus to prevent development of epilepsy. Unlike in hippocampus, the L-channel–NFAT pathway is slightly more complex in sympathetic neurons, in which L-channels underlie less than 15% of the total Ca^{2+} current, revealing both N- and L-type Ca^{2+} channel activity to be necessary for NFAT activation (Zhang & Shapiro, 2012). We have suggested a dual requirement model in these cells: L-channels serve as the critical initiating 'sensor' of activity and depolarization, and an elevated microdomain Ca_i^{2+} signal, which activates only AKAP–L-channel complex-bound CaN, despite the high abundance of CaN in neurons. Upon Ca^{2+} binding, CaN rapidly dissociates from AKAP79/150 complexes to dephosphorylate NFAT, because CaN affinity for

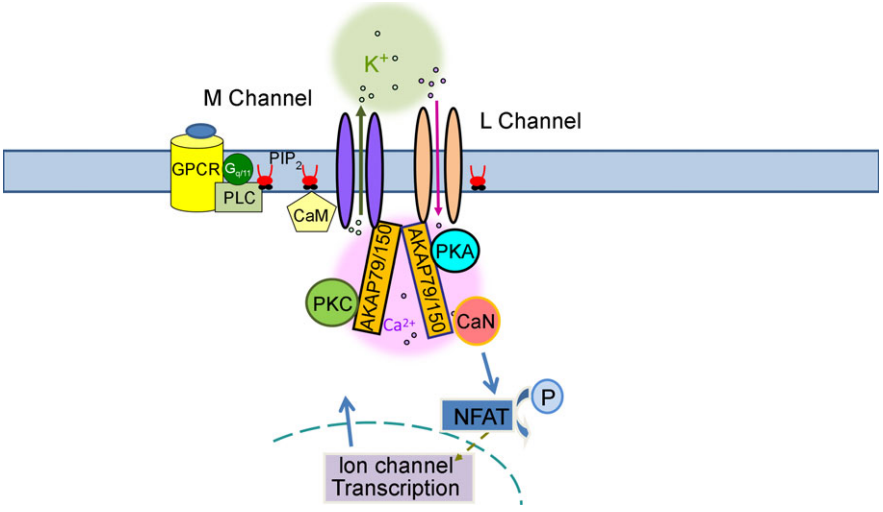

Figure 3. Model of putative AKAP79/150-orchestrated multi-channel protein complexes
AKAP79/150 expresses as a homodimer, and each protomer of AKAP79/150 binds to one PKA molecule, and could physically couple two distinct ion channel complexes together to form one large macro-molecular super-complex, such as containing the M-channel and L-channel we illustrate here. In this large multi-channel protein complex, channels are also likely to be functionally linked by AKAP79/150 (Dixon *et al.* 2012), via direct coupling of their gating, indirect coupling via Ca^{2+} or changes in [PIP_2], or more slowly via transcriptional regulation.

AKAP79/150 is relatively low ($K_D \approx 0.5\ \mu M$; Li *et al.* 2012). However, globally elevated $[Ca^{2+}]_i$ (mostly mediated by N-type Ca^{2+} channels in peripheral ganglia) is also required to keep CaN–NFAT activated during its import into the nucleus, This global Ca_i^{2+} elevation could probably come from various sources, not specifically from N-channels (Zhang & Shapiro, 2012).

Besides M-channels, large-conductance Ca^{2+}-activated BK_{Ca} channels have been discovered as a downstream target of AKAP-organized transcriptional signalling (Zhang & Shapiro, 2012; Nystoriak *et al.* 2014). In arterial myocytes, the same L-channel–AKAP–CaN complex as described above mediates down-regulation of BK_{Ca} channel $\beta 1$ subunits, contributing to BK_{Ca} channel remodelling and enhanced vasoconstriction during type II diabetes mellitus (Nystoriak *et al.* 2014). The critical questions are (1) whether NFATs pre-associate with the AKAP79/150–CaN–L-channel complex at the membrane and (2) whether CaN stays associated with NFAT during its import into the nucleus, perhaps thus explaining its delayed time to be transported into the nuclear compartment.

An additional layer of flexibility and complexity of AKAP signalling pathways comes from the recent discovery of the regulatory property of AKAP15, which binds competitively to the same LZ motif on the distal C-terminal domain of $Ca_V1.2$ channels as does AKAP79/150 and also seems to mediate PKA phosphorylation on L-channels (Fuller *et al.* 2014). AKAP15 and AKAP79/150 actions were shown to have different effects, dependent on their differential associations with regulatory proteins, notably CaN (Fuller *et al.* 2014). However, previous studies involving AKAP79/150 knockdown or knockouts that disrupt L-channel–AKAP–PKA–CaN complexes prevented specific PKA-, or CaN-mediated effects on L-channel function. Exciting studies from new transgenic mice with a specific deletion of the PKA binding segment or the CaN-binding PXIXIT motif on AKAP150, *AKAP150*ΔPKA or *AKAP150*ΔPIX, allow more specific probing of the physiological roles of AKAP-anchored PKA or CaN on L-channels. Surprisingly, removal of PKA from the $Ca_V1.2$–AKAP150 complex not only reduced basal L-channel phosphorylation and L-current density, but also impaired CaN-mediated NFAT activation and translocation to the nucleus (Dittmer *et al.* 2014; Murphy *et al.* 2014).

Interplay between AKAP79/150 anchored ion channels in super multi-channel complexes

Whether AKAP79/150-anchored proteins are physically coupled as larger super-complexes is an intriguing question to investigate, especially involving distinct ion channel complexes that shape neuronal activity (Fig. 3). The gating of individual $Ca_V1.2$ channels seems to be coupled (Navedo *et al.* 2010), probably via physical protein–protein interactions of $Ca_V1.2$ channels with one another at their carboxyl-tails by AKAP79/150 (Cheng *et al.* 2011), resulting in amplification of Ca^{2+} influx and increased excitation–contraction coupling in ventricular myocytes (Dixon *et al.* 2012). Recent structural and biochemical studies also suggest such a possibility, as AKAP79/150 is reported to express as homodimers coupled with a PKA homodimer and two CaN heterodimers (Gold *et al.* 2011). Since each AKAP79/150 protomer in such putative homodimers binds one PKA (Gold *et al.* 2011), they could also physically couple two distinct ion channel complexes to form one macro-molecular multi-channel complex. Recent use of super-resolution fluorescence imaging offering nanometer scale resolution has successfully revealed the molecular architecture of synapses, including organization of protein components of the pre-synaptic active zone and the postsynaptic density (Dani *et al.* 2010), and of actin–sepctrin–Na^+ channels organization in axons (Xu *et al.* 2012, 2013*a*). The next important step would be to elucidate the detailed mechanism of these actions in different excitable cells.

Conclusions

AKAP79/ is particularly crucial in its ability to form focally compartmentalized multi-protein signalling complexes in localized neuron structures, such as regulating pre- and postsynaptic plasticity (Sanderson & Dell'Acqua, 2011; Sanderson *et al.* 2012). Local perturbations of neuronal ion channel function by disrupted AKAP79/150 signalling complexes modulate synaptic transmission, and have been suggested to play a role in seizures, mental retardation, Alzheimer's disease and schizophrenia (Sanderson & Dell'Acqua, 2011; Esseltine & Scott, 2013). In this mini-review, we summarize the dual fast and slow regulation of ion channel activity by AKAP-organized protein complexes which includes rapidly altering the activity of ion channels already functioning in the membrane, and more slowly altering the population of expressed channels by transcriptional regulation in the nucleus, dependent on the activity of VGCCs. The highly dynamic feature of AKAP79/150 complexes upon neuronal stimulation suggests their novel ability to further fine tune the spatiotemporal resolution of localized signals. We also suggest that AKAP79/150 probably clusters together different ion channels that functionally interact, as a mechanism of feed-back inhibition, amplification or focusing of neuronal signals.

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Additional information

Competing interests

None declared.

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