Differential regulation of CaV1.2 channels by cAMP-dependent protein kinase bound to A-kinase anchoring proteins 15 and 79/150

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The CaV1.1 and CaV1.2 voltage-gated calcium channels initiate excitation-contraction coupling in skeletal and cardiac myocytes, excitation-transcription coupling in neurons, and many other cellular processes. Up-regulation of their activity by the β-adrenergic–PKA signaling pathway increases these physiological responses. PKA up-regulation of CaV1.2 activity can be reconstituted in a transfected cell system expressing CaV1.2Δ1800 truncated at the in vivo proteolytic processing site, the distal C-terminal domain (DCT; CaV1.2[1801–2122]), the auxiliary α2β and subunits of CaV1.2 channels, and A-kinase anchoring protein-15 (AKAP15), which binds to a site in the DCT. AKAP79/150 binds to the same site in the DCT as AKAP15. Here we report that AKAP79 is ineffective in supporting up-regulation of CaV1.2 channel activity by PKA, even though it binds to the same site in the DCT and inhibits the up-regulation of CaV1.2 channel activity supported by AKAP15. Mutation of the calcineurin-binding site in AKAP79 (AKAP79ΔPIX) allows it to support PKA-dependent up-regulation of CaV1.2 channel activity, suggesting that calcineurin bound to AKAP79 rapidly dephosphorylates CaV1.2 channels, thereby preventing their regulation by PKA. Both AKAP15 and AKAP79ΔPIX exert their regulatory effects on CaV1.2 channels in transfected cells by interaction with the modified leucine zipper motif in the DCT. Our results introduce an unexpected mode of differential regulation by AKAPs, in which binding of different AKAPs at a single site can competitively confer differential regulatory effects on the target protein by their association with different signaling proteins.

INTRODUCTION

Voltage-gated CaV (CaV) channels initiate excitation-contraction coupling in muscle cells, excitation-transcription coupling in neurons, and many other physiological events (Reuter, 1979; Catterall, 1991; Bers, 2002; West et al., 2002). In skeletal and cardiac muscle, up-regulation of the activity of CaV1.1 and CaV1.2 channels increases contractile force in response to activation of the β-adrenergic signaling pathway in the fight or flight response (Reuter, 1983; Tsien et al., 1986; Catterall, 2000). In neurons, activation of the dopamine and β-adrenergic signaling pathways increases CaV1.2 channel activity and modulates gene transcription and synaptic plasticity (Lovingier, 2010; Gerfen and Surmeier, 2011; Qian et al., 2012). β-Adrenergic receptors activate adenylyl cyclase, increase cAMP, activate cAMP-dependent protein kinase (PKA), and phosphorylate CaV1.1 and CaV1.2 channels (Reuter, 1983; Tsien et al., 1986; Catterall, 2000). Targeting PKA to specific subcellular compartments or substrates by binding to A-kinase anchoring proteins (AKAPs) exerts spatiotemporal control over these regulatory processes (Wong and Scott, 2004).

CaV1.1 and CaV1.2 channels form autoinhibitory signaling complexes, which are essential for regulation of their activity by the PKA pathway (Hulme et al., 2004; Catterall, 2010). Their pore-forming α1 subunits are proteolytically processed in vivo near the center of their large intracellular C-terminal domains (De Jongh et al., 1989, 1991, 1996; Hell et al., 1996). The membrane-anchored AKAP15/18 (Gray et al., 1997, 1998; Fraser et al., 1998) binds to the distal C-terminal domain (DCT; CaV1.2[1801–2122]) of these channels via a modified leucine zipper motif (Hulme et al., 2002, 2003). The DCT and AKAP binding are required for regulation of CaV1.1 and CaV1.2 channels by PKA in skeletal and cardiac myocytes (Gray et al., 1998; Hulme et al., 2002, 2003; Ganesan et al., 2006; Fu et al., 2011). Moreover, the proteolytically cleaved DCT binds to the remainder of the CaV1.1 and CaV1.2 channels by interaction with a site in the proximal C-terminal domain (Hulme et al., 2005, 2006) and is a potent autoinhibitor of the activity.
of CaV1.2 channels when coexpressed in nonmuscle cells (Hulme et al., 2006). Activation of protein phosphorylation by PKA increases ion conductance activity by relieving the autoinhibitory effect of the DCT (Fuller et al., 2010).

Regulation of CaV1.2 channels by PKA has been reconstituted by coexpression of the components of this autoinhibitory signaling complex in transfected cells (Fuller et al., 2010). CaV1.2Δ1800 truncated at the site of in vivo proteolytic processing (Emrick et al., 2010) and the DCT composed of CaV1.2[1801–2122] interact with each other when expressed as separate proteins, and the DCT markedly inhibits CaV1.2 channel activity (Hulme et al., 2006; Fuller et al., 2010). Coexpression of these two components of the α1 subunit as separate proteins together with the auxiliary α2δ and β subunits of CaV1.2 channels and AKAP15 yields an autoinhibited CaV1.2 signaling complex whose activity can be increased three- to fourfold by activation of adenylyl cyclase in transfected cells (Fuller et al., 2010). Normal regulation of basal activity requires phosphorylation of Ser1700 and Thr1704, located at the interface between the DCT and the proximal C-terminal domain, and up-regulation of CaV1.2 channel activity requires PKA phosphorylation of Ser1700 (Fuller et al., 2010). Cardiac myocytes from mice in which these sites are mutated to Ala have reduced basal L-type Ca2+ current and impaired up-regulation by β-adrenergic agonists, confirming the crucial role of this regulatory mechanism in vivo (Fu et al., 2013). AKAP15 also binds to CaV1.2 channels in brain neurons (Marshall et al., 2011), and AKAP79/150 binds to CaV1.2 channels in both brain and cardiac muscle (Gao et al., 1997; Hall et al., 2007). Both of these AKAPs are involved in regulation of gene expression in response to activation of CaV1.2 channels in neurons (Oliveria et al., 2007; Marshall et al., 2011). The effects of AKAP79 on gene transcription in neurons are mediated by the Ca2+-regulated phosphoprotein phosphatase calcineurin, which binds directly to AKAP79 (Oliveria et al., 2007). Like AKAP15, AKAP79 binds to CaV1.2 channels via the modified leucine zipper motif in the DCT (Oliveria et al., 2007). Therefore, it is of great interest to explore PKA regulation of CaV1.2 channels mediated via AKAP79 compared with AKAP15 in our reconstituted regulatory system. Here we report strikingly different regulatory properties of these two AKAPs, which depend on binding of the phosphoprotein phosphatase calcineurin by AKAP79. Our results introduce an unexpected mode of differential regulation by AKAPs, in which different AKAPs can compete for binding at a single site and confer differential regulatory effects on the target protein by association with different signaling proteins.

MATERIALS AND METHODS

cDNA constructs

Constructs used in this study include rabbit α1.2a, rat β2b, rabbit α2δ1, AKAP15, AKAP79, PKA-Cα, and PKA-RHα in pcDNA3 (Fuller et al., 2010). CaV1.2 leucine zipper motif triple mutant (I2073A, F2080A, I2087A) was constructed using PCR overlap extension. Construction of AKAP244 was previously described (Hulme et al., 2002). AKAP79pix was provided by J.D. Scott (University of Washington, Seattle, WA). The mutant sequence, orientation, and reading frame of all constructs were confirmed by DNA sequencing.

Cell culture and transfection

Human embryonic kidney tsA201 cells were cultured in DMEM/ Ham’s F12 supplemented with 10% FBS and 100 U/ml penicillin and streptomycin. Cells were grown to ~70% confluence in 10% CO2 and transiently transfected with cDNAs encoding α1.2αa truncated at Ala1800 (CaV1.2Δ1800), β2b, and α2δ1 subunits at a 1:1:1 molar ratio using the FuGENE 6 method (Roche). Wild-type or mutant DCT constructs composed of CaV1.2[1801–2271] were transfected with CaV1.2Δ1800 using a molar ratio of 0.75:1 (DCT/ CaV1.2Δ1800). In addition, cDNA encoding eGFP in the pcDNA3 vector was added at a molar ratio of 0.1:1 to each transfection mixture as an indicator of transfection efficiency.

Electrophysiology

24 h after transfection, cells were plated at low density, and recordings were made 38–48 h after transfection using the whole-cell configuration of the patch clamp technique. Patch pipettes (1.5–2 MΩ) were pulled from micropipette glass (VWR Scientific) and fire-polished. Currents were recorded with an Axopatch 200B amplifier (Axon Instruments Inc.) and sampled at 5 kHz after anti-alias filtering at 2 kHz. Data acquisition and command potentials were controlled by either pCLAMP or HEKA Pulse software, and data were stored for later offline analysis. Voltage protocols were delivered at 10-s intervals, and leak and capacitive transients were subtracted using a P/4 protocol. Approximately 80% of series resistance was compensated with the patch clamp circuitry. The extracellular solution contained (mM) 150 Tris, 10 glucose, 1 MgCl2, and 10 BaCl2 (adjusted to pH 7.4 with CH3SO3). The intracellular solution contained (mM) 135 CsCl2, 10 EGTA, 1 MgCl2, 4 MgATP, and 10 HEPES (pH 7.3, adjusted with CsOH).

Analysis of electrophysiological recordings of CaV1.2 channels

Current-voltage relationships from peak inward Ba2+ currents were normalized to gating charge (Q) to correct for variation in protein abundance. Gating currents result from the voltage-driven movement of gating charges as conformational changes occur preceding channel opening and are independent of channel unitary conductance and open probability (P0). Gating charge movement was measured as the integral of the gating current transient at the reversal potential of the ionic current (Fig. S1). The reversal potential was determined by applying a series of test pulses at 10-s intervals from the holding potential of −80 mV to potentials between 60 and 80 mV in 2-mV increments. The ionic current that flows upon repolarization (the tail current) gives a functional readout proportional to the number of open channels, the single channel conductance, and channel Po at the end of the depolarizing step. By comparing the ionic and gating currents we determined the efficiency of coupling of the charge movement of the voltage sensors to the subsequent opening of the pore by calculating the ratio of tail current to gating charge (tail current [nA]/integrated gating charge [pC]). Tail currents were recorded after repolarization to −50 mV after each test pulse. All data are expressed as means ± SEM of n cells. Bar graphs are presented for coupling ratio data in the figures, and scatter plots containing all of the individual cell values are presented for representative experiments in Fig. S2. Statistical significance was tested with Student’s t test for pairwise analysis and ANOVA followed by Dunnett’s test for comparison of multiple conditions.
Differential regulation of CaV1.2 channels by association with AKAP15 versus AKAP79

Neurons, myocytes, and other excitable cells express a broad array of AKAPs, which are involved in many aspects of cell signaling (Logue and Scott, 2010). AKAP15 and AKAP79 are both expressed in nerve and muscle cells and interact with a common regulatory site on CaV1.2 channels in those cell types (Gray et al., 1997, 1998; Hulme et al., 2003, 2006; Hall et al., 2007; Oliveria et al., 2007; Marshall et al., 2011). We used reconstitution of CaV1.2 regulation in transfected cells to compare the effects of these two AKAPs on regulation of CaV1.2 channels via the PKA pathway, and we recorded barium currents (I_Ba) conducted by CaV1.2 channels to minimize activation of Ca^2+-dependent regulatory processes. Human embryonic kidney tsA-201 cells were cotransfected with CaV1.2Δ1800, DCT, and CaV1.2 channel auxiliary subunits, plus either AKAP15 or AKAP79 (Fig. 1). Cells transfected with CaV1.2Δ1800 without DCT conducted high levels of I_Ba upon depolarization in whole-cell voltage clamp (Fig. 1, A [top] and B). In contrast, cells transfected with CaV1.2Δ1800 + DCT had much lower levels of I_Ba, reflecting the autoinhibitory effect of the DCT (Fig. 1, A [top] and B). The autoinhibitory effect of the DCT was also observed in measurements of the coupling ratio of ion channel opening to gating charge movement, which was calculated from measurements of gating charge movement at the reversal potential and measurement of gating charge at that potential. Fig. 1, A–C, illustrates the coupling ratio data for individual cells that contribute to the means for representative experiments. Online supplemental material is available at http://www.jgp.org/cgi/content/full/jgp.201311075/DCT.

RESULTS

Differential regulation of CaV1.2 channels by association with AKAP15 versus AKAP79

If AKAP15 and AKAP79 both interact with the AKAP-binding domain in the DCT of CaV1.2 channels, they should compete with each other for binding to that regulatory site and PKA regulation via AKAP15 should be inhibited by coexpression of AKAP79. To examine this point, we expressed CaV1.2Δ1800 + DCT with AKAP15 + AKAP79 and measured regulation by activation of adenylyl cyclase with forskolin (Fig. 2). The results show that AKAP79 does inhibit PKA regulation of CaV1.2Δ1800 + DCT coexpressed with AKAP15, as measured by the amplitude of I_Ba and the coupling ratio (Fig. 2). One potential mechanism of competitive interaction between AKAP15 and AKAP79 would be competitive binding of PKA by AKAP79, which could potentially deplete the cellular pool of PKA. To rule out this possibility, we overexpressed PKA in the presence of the two AKAPs (Fig. 2 C). Even under these conditions, expression of AKAP79 substantially reduced PKA regulation of CaV1.2Δ1800 + DCT via AKAP15 (Fig. 2 C). Together, these results demonstrate competitive regulation of CaV1.2 channels by AKAP15 and AKAP79, dependent on their binding to the AKAP-binding domain in the DCT.

Differential regulation requires calcineurin association with AKAP79

One potential mechanism that could contribute to differential PKA regulation via AKAP15 and AKAP79 is the ability of AKAP79 to bind other signaling molecules and bring them into close association with CaV1.2 channels (Logue and Scott, 2010). In particular, AKAP79 binds the Ca^2+-regulated phosphoprotein phosphatase calcineurin (Coghlan et al., 1995; Oliveria et al., 2007), which could dephosphorylate CaV1.2 channels and reduce their up-regulation by PKA. Calcineurin binds to the PXIXIT motif on AKAP79 (Dell’Acqua et al., 2002; Oliveria et al., 2007). Therefore, we examined regulation of CaV1.2Δ1800 + DCT coexpressed with AKAP79 with the PXIXIT site deleted (AKAP79ΔPIX). Under these conditions, forskolin treatment increased the activity of CaV1.2Δ1800 + DCT coexpressed with AKAP79ΔPIX as cDNA ratios did not result in an increase of either I_Ba or coupling ratio (Fig. 1, A–C), even though AKAP79 is known to bind to the AKAP-binding site in the DCT of CaV1.2 channels (Oliveria et al., 2007). To assure that PKA was not limiting in these experiments, we overexpressed PKA as described previously (Fuller et al., 2010) and conducted a similar series of experiments (Fig. 1, D and E). Forskolin had no effect in the absence of any AKAP or in the presence of AKAP79, in contrast to the substantial increase in I_Ba and coupling ratio in the presence of AKAP15. These results reveal striking differential regulation of CaV1.2 channels via the PKA pathway dependent on their association with AKAP15 versus AKAP79.
effectively as CaV1.2Δ1800 + DCT coexpressed with AKAP15, when measured as the amplitude of I_{Ba} or the coupling ratio (Fig. 3, A–C). Overexpression of PKA further increased I_{Ba} and coupling ratio for CaV1.2Δ1800 + DCT coexpressed with either AKAP79ΔPIX or AKAP15 (Fig. 3, D and E). Under experimental conditions similar to ours, Oliveria et al. (2007) found that competing peptide inhibitors of the binding of calcineurin to CaV1.2 channels gave similar results as the ΔPIX mutation, indicating that the effects of this mutation are caused by inhibition of calcineurin binding and not by a more global conformational change in AKAP79. Moreover,
our experiments show a gain of function effect of AKAP79ΔPIX, fully restoring its ability to support PKA regulation at the same level as AKAP15 (Fig. 3 E). Complete restoration of the activity of AKAP79 to the equivalent of AKAP15 would not be expected for a mutation-induced, nonspecific conformational change in AKAP79. Therefore, our results implicate binding of calcineurin to AKAP79 as the primary reason for its differential regulation of CaV1.2 channels via the PKA pathway.

Although calcineurin is strongly Ca2+ regulated, it has a significant basal activity (Stewart et al., 1982; Perrino et al., 1992; Stemmer and Klee, 1994). In our experiments, we measure Ba2+ currents in low extracellular Ca2+ and we chelate intracellular Ca2+ with EGTA; therefore, it is likely that the basal activity of calcineurin in the presence of entering Ba2+ is sufficient to oppose PKA regulation in this experimental system. Consistent with this conclusion, we found that treatment with 5 µM cyclosporin A, which blocks up-regulation of calcineurin activity by Ca2+ without affecting basal activity (Fruman et al., 1992), did not significantly increase Ba2+ currents (P = 0.33). The mechanism of this effect of basal calcineurin activity on PKA regulation of CaV1.2 channels is considered further in the Discussion.

AKAP15 and AKAP79ΔPIX require the modified leucine zipper motif in the DCT

AKAP15 binds to skeletal muscle CaV1.1 channels and cardiac CaV1.2 channels via a modified leucine zipper interaction between a heptad repeat of hydrophobic residues in the AKAP-binding domain in the DCT and a similar heptad repeat of two Leu residues in AKAP15, and this modified leucine zipper interaction is required for PKA regulation of CaV1.2 channels in skeletal and cardiac myocytes (Hulme et al., 2002, 2003). To confirm that this leucine zipper interaction is also required for PKA regulation of CaV1.2Δ1800 + DCT in our reconstituted regulatory system in transfected tsA-201 cells, we tested the regulatory effects of AKAP15LZM, in which the two Leu residues in heptad repeat in AKAP15 are mutated to Ala (Fig. 4, A–C). Our results show that AKAP15LZM is completely ineffective in supporting PKA regulation of CaV1.2Δ1800 + DCT, as measured by increased I_{Ba} or increased coupling ratio (Fig. 4, A–C). In complementary experiments, we substituted Ala for the three hydrophobic residues in the heptad repeat that forms the AKAP-binding domain in the DCT of CaV1.2 channels to create the triple mutant DCT LZM. Coexpression of CaV1.2Δ1800 + DCT LZM with AKAP15 also resulted in loss of regulation via the PKA pathway (Fig. 4, D–F). These results confirm that PKA regulation via AKAP15 in our reconstituted system requires interaction of the leucine zipper motif in AKAP15 with the complementary modified leucine zipper motif in the DCT of CaV1.2 channels.

Although AKAP79 is known to interact with CaV1.2 channels via the modified leucine zipper motif in the DCT (Hall et al., 2007; Oliveria et al., 2007), the role of this interaction in up-regulation of ion conductance activity of CaV1.2 channels has not been directly tested. Our
ability to reconstitute PKA regulation of Ca\(_{\text{v1.2}}\) channels by coexpression with AKAP79\(\Delta\)PIX in transfected cells now allows a direct test of its site of interaction using the DCT\(\_{\text{LM}}\) mutant. We found that Ca\(_{\text{v1.2}}\Delta1800 +\) DCT\(\_{\text{LM}}\) coexpressed with AKAP79\(\Delta\)PIX was not up-regulated by activation of adenylyl cyclase with forskolin (Fig. 4, G and H). These results show directly that the regulatory effects of AKAP79\(\Delta\)PIX, as well as those of AKAP15, require interaction with the modified leucine zipper motif in the DCT of Ca\(_{\text{v1.2}}\) channels.

**DISCUSSION**

Reconstitution of regulation of Ca\(_{\text{v1.2}}\) channels by the PKA signaling pathway

The results presented here further establish the relevance of our reconstitution system for studies of regulation of Ca\(_{\text{v1.2}}\) channels by the PKA signaling pathway. Coexpression of different members of the autoinhibitory signaling complex formed by Ca\(_{\text{v1.2}}\) channels allows their differential regulatory properties to be directly determined and compared with the properties of Ca\(_{\text{v1.2}}\) channels expressed with AKAP15. Using this approach, we have found that AKAP79 confers strikingly different regulation from AKAP15. These results imply that differential expression and localization of AKAP15 and AKAP79 can lead to differential regulation of Ca\(_{\text{v1.2}}\) channels in different cell types and subcellular compartments. Our results highlight the importance of binding of the Ca\(^{2+}\)-regulated phosphoprotein phosphatase calcineurin in determining the regulatory properties of AKAP79. A previous study has demonstrated its role in regulation of basal activity of Ca\(_{\text{v1.2}}\) channels and in regulation of gene expression via ability to reconstitute PKA regulation of Ca\(_{\text{v1.2}}\) channels by coexpression with AKAP79\(\Delta\)PIX in transfected cells now allows a direct test of its site of interaction using the DCT\(\_{\text{LM}}\) mutant. We found that Ca\(_{\text{v1.2}}\Delta1800 +\) DCT\(\_{\text{LM}}\) coexpressed with AKAP79\(\Delta\)PIX was not up-regulated by activation of adenylyl cyclase with forskolin (Fig. 4, G and H). These results show directly that the regulatory effects of AKAP79\(\Delta\)PIX, as well as those of AKAP15, require interaction with the modified leucine zipper motif in the DCT of Ca\(_{\text{v1.2}}\) channels.

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entry of $\text{Ca}^{2+}$ through CaV1.2 channels (Oliveria et al., 2007). Our results provide direct evidence for an important role for calcineurin bound to AKAP79 in opposing up-regulation of CaV1.2 channel function by the PKA pathway. A recent study shows that AKAP79 also binds phosphoprotein phosphatase-1 (Le et al., 2011), which may contribute additional modes of differential regulation of CaV1.2 channels and other PKA signaling targets.

Differential regulation of CaV1.2 channels by PKA bound to different AKAPs

Most cells express several different AKAPs, which have been shown to participate in many different cell signaling pathways (Logue and Scott, 2010). Thus, differential regulation of distinct signaling pathways by different AKAPs is well established. Our results with CaV1.2 channels add a new perspective on the potential molecular mechanisms for differential regulation by AKAPs by

Figure 4. AKAP15 and AKAP79ΔPIX regulate CaV1.2 channels through a modified leucine zipper motif located in the DCT. (A) Representative $I_{\text{Ba}}$ through CaV1.2Δ1800 and CaV1.2Δ1800 + DCT channels coexpressed with cDNA ratios of 0.003:1 WT AKAP15 or AKAP15ΔZM in the absence or presence of 5 µM forskolin (Fsk) elicited by a test pulse to 10 mV from a holding potential of −80 mV. (B) Mean current-voltage relationships for CaV1.2Δ1800 and CaV1.2Δ1800 + DCT channels coexpressed with cDNA ratios of 0.003:1 WT AKAP15 or AKAP15ΔZM and 5 µM forskolin. Dashed black line indicates mean coupling ratio for unstimulated CaV1.2Δ1800 + DCT with AKAP15ΔZM. **, $P < 0.01$ versus control. (C) Coupling ratio ($nA/pC$) for CaV1.2Δ1800 and CaV1.2Δ1800 + DCT channels with WT AKAP15 or AKAP15ΔZM without and with 5 µM forskolin. Dashed black line indicates mean coupling ratio for unstimulated CaV1.2Δ1800 + DCT with AKAP15ΔZM. **, $P < 0.01$ versus control. (D) Representative $I_{\text{Ba}}$ through CaV1.2Δ1800 channels coexpressed with either WT DCT or DCTΔZM and a cDNA ratio of 0.003:1 AKAP15 without or with 5 µM forskolin. (E) Mean current-voltage relationships for CaV1.2Δ1800 channels coexpressed with WT DCT or DCTΔZM and a cDNA ratio of 0.003:1 WT AKAP15 without or with 5 µM forskolin. (F) Coupling efficiency ($nA/pC$) for CaV1.2Δ1800 channels in E. Dashed black line indicates mean coupling ratio for unstimulated CaV1.2Δ1800 + DCTΔZM with AKAP15ΔZM. **, $P < 0.01$ versus control. (G) Representative $I_{\text{Ba}}$ through CaV1.2Δ1800 channels coexpressed with WT DCT or DCTΔZM and 0.01:1 AKAP79ΔPIX in the absence or presence of 5 µM forskolin. (H) Mean current-voltage relationships for CaV1.2Δ1800 channels with either WT DCT or DCTΔZM and a cDNA ratio of 0.01:1 AKAP79ΔPIX, and 5 µM forskolin. (B, C, E, F, and H) Error bars are SEM. (I) Coupling ratio ($nA/pC$) for CaV1.2Δ1800 channels in H. Dashed black line indicates mean coupling ratio for unstimulated CaV1.2Δ1800 + DCTΔZM with AKAP79ΔPIX. $n$ values and means ± SEM are indicated. **, $P < 0.01$ versus control. Significance was determined by ANOVA.
revealing that multiple AKAPs can bind at a single regulatory site on their common target protein and have differential effects on the same cell signaling pathway that are mediated by their associations with different regulatory proteins. AKAP15 and AKAP79 have previously been shown to interact with the same, short modified leucine zipper motif, which is required for their binding and support of PKA regulation (Hulme et al., 2002, 2003; Oliveria et al., 2007). However, it was unknown whether interaction with this common site would cause functional competition between the two proteins. We found that AKAP15 and AKAP79 do indeed compete functionally when coexpressed with CaV1.2 channels in the autophosphorylating complex. Moreover, this functional competition is caused by the ability of AKAP79 to bind calcineurin. Because different AKAPs bind many different kinases, phosphoprotein phosphatases, and other signaling proteins (Logue and Scott, 2010), this form of functional competition and differential regulation of target proteins by different AKAPs acting at a common binding site would provide a broad range of regulatory options controlled by expression and localization of AKAPs. These findings add an additional layer of flexibility and complexity to cell signaling pathways in which AKAPs organize multiple regulatory proteins.

Our results demonstrating functional competition among AKAPs interacting with the same site on CaV1.2 channels take on additional significance in light of recent mouse genetic studies of AKAP regulation of the heart (Jones et al., 2012). Deletion of both AKAP15 and AKAP79 in mice is not sufficient to prevent β-adrenergic up-regulation of CaV1.2 channel activity by isoproterenol in ventricular myocytes (Jones et al., 2012). Because β-adrenergic stimulation in ventricular myocytes requires AKAP anchoring at the AKAP-binding domain on CaV1.2 channels (Hulme et al., 2003), these mouse genetic results imply that one or more additional AKAPs besides AKAP15 and AKAP79 can mediate β-adrenergic stimulation of CaV1.2 channels in ventricular myocytes through interaction with the same site. Thus, functional competition for regulation of CaV1.2 channels by AKAPs likely extends to at least one more, yet-unidentified AKAP. The levels of expression and affinity and the different regulatory properties of these AKAPs will determine which one is dominant in regulating CaV1.2 channels and therefore will define the overall pattern of regulation of channel activity. Changes in expression of these AKAPs in different physiological and/or pathological states may be important determinants of the activity of CaV1.2 channels.

Regulation of CaV1.2 channels by calcineurin bound to AKAP79

Differential regulation of CaV1.2 channels by AKAPs might reflect altered interactions between the channel and AKAP or differential interactions of the bound AKAP with other signaling proteins. Our results with AKAP79 show that its binding of calcineurin is responsible for its inability to support PKA regulation of CaV1.2 channels. Surprisingly, the basal phosphatase activity of calcineurin is sufficient for this regulatory effect. Our measurements are made using Ca2+ as the permeant ion, and intracellular Ca2+ is strongly buffered with EGTA in the recording pipette. Therefore, it is unlikely that calcineurin is substantially up-regulated by Ca2+ binding in our experiments. Calcineurin has a significant basal activity, which is ~0.25–1% of its maximal activity when fully activated by Ca2+ and calmodulin in biochemical assays in solution (Stewart et al., 1982; Perrino et al., 1992; Stemmer and Klee, 1994). The rate of dephosphorylation of phosphoprotein substrates depends on their local concentration in the vicinity of the phosphatase, and tethering of calcineurin directly to the DCT of CaV1.2 channels would increase the local concentration of its substrate site at Ser1700-P by hundreds or thousands of fold. Evidently, the effect of proximity afforded by binding to AKAP79 allows effective dephosphorylation of the CaV1.2 channel by the basal activity of calcineurin at a rate that is comparable with or greater than the rate of phosphorylation by PKA, preventing accumulation of phosphorylated CaV1.2 channels and resulting in functional competition between AKAP15 and AKAP79 at their common binding site. In a cardiac myocyte, increases of cAMP near CaV1.2 channels may be faster and larger because of localized signaling; therefore, activation of calcineurin by Ca2+ entering through CaV1.2 channels may be required to return the activity of these channels to the basal level in vivo. These considerations further emphasize the importance of a signaling complex for regulation of CaV1.2 channels in vivo.

Regulation of CaV1.2 channels in different tissues

CaV1.2 channels conduct L-type Ca2+ currents in several different cell types. In skeletal and cardiac myocytes, up-regulation of CaV1.2 channel activity in response to activation of the β-adrenergic signaling pathway contributes to the increase in contractile force during the fight or flight response (Reuter, 1983; Tsien et al., 1986; Catterall, 1991). In brain neurons, CaV1.2 channels are involved in synaptic plasticity on the postsynaptic side of the synapse, and up-regulation of their activity by the β-adrenergic and dopaminergic signaling pathways enhances synaptic transmission (Davare et al., 2001; Young and Yang, 2004; Hall et al., 2007). In endocrine cells, CaV1.2 channels mediate Ca2+ entry that triggers secretion of hormones, and regulation by the PKA signaling pathway is an important regulator of hormone release (Baldelli et al., 2004; Yang and Berggren, 2006). AKAP15 and AKAP79 can regulate CaV1.2 channels in skeletal and cardiac myocytes (Hulme et al., 2002, 2003) and brain neurons (Hall et al., 2007; Marshall et al., 2011), and AKAPs are also implicated in control of hormone
secretion (Lester et al., 2001; Yang and Berggren, 2006). Our results presented here imply that competitive binding of AKAP15, AKAP79, and potentially other AKAPs at the AKAP-binding domain on CaV1.2 channels can transform the regulatory responses of CaV1.2 channels to the PKA signaling pathway and potentially to other intracellular signaling pathways and thereby can fine-tune the regulation of muscle contraction, synaptic transmission, and hormone secretion.

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