The PDZ Motif of the α_{1C} Subunit Is Not Required for Surface Trafficking and Adrenergic Modulation of Ca_{V}1.2 Channel in the Heart*

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Lin Yang¹, Alexander Katchman¹, Richard L. Weinberg², Jeffrey Abrams¹, Tahmina Samad¹, Elaine Wan³, Geoffrey S. Pitt⁵, and Steven O. Marx¹,³

From the ¹Division of Cardiology, Departments of Medicine and Pharmacology, College of Physicians and Surgeons, Columbia University, New York, New York 10032 and the ²Division of Cardiology, Department of Medicine, Duke University Medical Center, Durham, North Carolina 27710

Background: The mechanisms responsible for Ca_{V}1.2 regulation by the α_{1C} C terminus are unknown. Results: Trafficking, basal function, and adrenergic modulation of Ca_{V}1.2 were not altered in cardiomyocytes of transgenic mice expressing PDZ-deleted α_{1C}.

Conclusion: PDZ-mediated interactions are not required for Ca_{V}1.2 trafficking and function in the heart.

Significance: The regulation of Ca_{V}1.2 by auxiliary proteins does not depend on the PDZ ligand motif in the heart.

Voltage-gated Ca^{2+} channels play a key role in initiating muscle excitation-contraction coupling, neurotransmitter release, gene expression, and hormone secretion. The association of Ca_{V}1.2 with a supramolecular complex impacts trafficking, localization, turnover, and, most importantly, multifaceted regulation of its function in the heart. Several studies hint at an important role for the C terminus of the α_{1C} subunit as a hub for multidimensional regulation of Ca_{V}1.2 channel trafficking and function. Recent studies have demonstrated an important role for the four-residue PDZ binding motif at the C terminus of α_{1C} in interacting with scaffold proteins containing PDZ domains, in the subcellular localization of Ca_{V}1.2 in neurons, and in the efficient signaling to cAMP-response element-binding protein in neurons. However, the role of the α_{1C} PDZ ligand domain in the heart is not known. To determine whether the α_{1C} PDZ motif is critical for Ca_{V}1.2 trafficking and function in cardiomyocytes, we generated transgenic mice with inducible expression of an N-terminal FLAG epitope-tagged dihydropyridine-resistant α_{1C} with the PDZ motif deleted (ΔPDZ). These mice were crossed with α-myosin heavy chain reverse transcriptional transactivator transgenic mice, and the double-transgenic mice were fed doxycycline. The ΔPDZ channels expressed, trafficked to the membrane, and supported robust excitation-contraction coupling in the presence of nisoldipine, a dihydropyridine Ca^{2+} channel blocker, providing functional evidence that they appropriately target to dyads. The ΔPDZ Ca^{2+} channels were appropriately regulated by isoproterenol and forskolin. These data indicate that the α_{1C} PDZ motif is not required for surface trafficking, localization to the dyad, or adrenergic stimulation of Ca_{V}1.2 in adult cardiomyocytes.

Ca_{V}1.2 has a key role in cardiac muscle excitation-contraction (E-C)⁴ coupling (1) and in determining the plateau phase of the action potential (2). The association of Ca_{V}1.2 with a macromolecular complex affects the trafficking and localization of Ca_{V}1.2 to the dyad in cardiomyocytes, its turnover, and, perhaps more importantly, multiple aspects of its function (3–5). In addition to the Ca_{V}1.2 subunits, α_{1C}, β, α_{2}/δγ, and γ (6), the complex includes calmodulin (7, 8), kinases (9, 10), phosphatases (11, 12), scaffold proteins (13, 14), BIN1 (15), caveolin-3 (16), and the β2-adrenergic receptor (9).

Knockin mice expressing α_{1C} truncated either at Gly¹⁷⁹⁶ or Asp¹⁹⁰⁴ displayed a dramatic reduction in Ca_{V}1.2 surface expression and current (I_{Ca,L}) in cardiomyocytes and exhibited cardiac failure and perinatal death (17, 18), suggesting a prominent role of the α_{1C} distal C terminus (residues 1796–2171) in Ca_{V}1.2 trafficking and function in the heart. Furthermore, the truncated α_{1C}¹⁹⁰⁴ that did make it to the surface in knockin mouse neonatal cardiomyocytes was insensitive to β-adrenergic modulation, suggesting a role of the distal C terminus of α_{1C} in mediating sympathetic nervous system activation of the Ca^{2+} channel in the heart (17, 18). These studies identify an important role for the distal C terminus of α_{1C} in regulating Ca_{V}1.2 trafficking and function in the heart, but the precise mechanisms and determinants underlying its role are unknown. Binding sites for several proteins in the Ca_{V}1.2 macromolecular complex have been mapped to discrete regions in the α_{1C} C terminus, although the role of many of these putative interaction sites in modulating Ca_{V}1.2 trafficking and function have not yet been tested in cardiomyocytes (12–14, 19–21).

—from the text—

* The abbreviations used are: E-C, excitation-contraction; pWT, pseudo-WT; MHC, myosin heavy chain; DHP, dihydropyridine; rtTA, reverse transcriptional transactivator.
PDZ domains are protein interaction motifs that bind to specific C-terminal sequences of their interacting proteins. PDZs are relatively promiscuous interaction domains that may have specificity for more than one target protein. The pore-forming subunits of both CaV1.2 and CaV1.3, two subtypes of L-type Ca\(^{2+}\) channels, contain evolutionarily conserved class 1 PDZ domain-binding C-terminal motifs (Fig. 1). To efficiently activate cAMP-response element-binding protein and gene expression in hippocampal neurons, interactions between the \(\alpha_{1C}\) subunit and proteins with a PDZ domain have been shown to be required (21), although the PDZ motif is not required for correct subcellular distribution or membrane expression of CaV1.2 in dendrites (22). The role of the PDZ ligand motif of CaV1.2, therefore, differs from that of CaV1.3, which depends on its PDZ motif for association with Shank and insertion into the postsynaptic membrane (23), and CaV2.2, which depends on its PDZ motif for insertion into the presynaptic space (24). The PDZ of \(\alpha_{1C}\) has also been shown to interact with proteins containing PDZ domains, such as enigma homolog 1 (25), which is a protein kinase D1-scaffolding protein, Cypher/ZASP (26), which is an protein kinase A anchoring protein, membrane-associated guanylate kinase-inverted proteins, Na\(^+\)/H\(^+\) exchanger regulatory factor 1/2, and neuronal nitric oxide synthase (nNOS) (27). In the heart, the PDZ-dependent association of \(\alpha_{1C}\) with Cypher/ZASP has been proposed to facilitate \(\beta\)-adrenergic-mediated phosphorylation of CaV1.2, and deletion of the \(\alpha_{1C}\) C-terminal PDZ motif significantly impaired PKA phosphorylation of Ser\(^{1925}\) of heterologously expressed CaV1.2 (26).

The roles of the \(\alpha_{1C}\) PDZ ligand motif in modulating trafficking, E-C coupling, and \(\beta\)-adrenergic modulation of Ca\(^{2+}\) current has not been directly tested in the heart. We determined the role of the PDZ motif in native cardiomyocytes by creating a transgenic mouse expressing an \(\alpha_{1C}\) subunit harboring a deletion of the C-terminal PDZ ligand motif.

**EXPERIMENTAL PROCEDURES**

**Reagents**—Nisoldipine (Santa Cruz Biotechnology) was dissolved weakly at a concentration of 30 mM in ethanol, protected from light, and diluted with ethanol on the day of the experiment to 3 mM. The final dilution of nisoldipine to 300 nM was in the extracellular recording solution. All other chemicals were acquired from Sigma.

**Animals**—The pseudo-WT (pWT) \(\alpha_{1C}\) and the \(\Delta\)PDZ (C-terminal four amino acid residues deleted) constructs were generated by fusing the rabbit Cacna1c cDNA (accession no. X15539) to the clone 26 vector containing the modified murine \(\alpha\)-myosin heavy chain (MHC), tetracycline-inducible promoter (“responder” line) vector (a gift from Drs. Jeffrey Robbins and Jeffrey Molkentin) (28, 29). The \(\alpha_{1C}\) subunit was engineered to be dihydropropyridine (DHP)-insensitive with the substitutions T1066Y and Q1070M (30, 31), and a 3× FLAG epitope was ligated in-frame to the N terminus of \(\alpha_{1C}\). Transgenic founder mice were identified with genomic DNA utilizing polymerase chain reactions using the following PCR primers: forward within clone 26 vector, CTT CCA GCC CTC TCT TWC TC; reverse \(\alpha_{1C}\), CAG CTG CGT TGG CAT TCA TGT TG. Transgenic-positive mice were bred with cardiac-specific (\(\alpha\)MHC), doxycycline-regulated, codon-optimized reverse transcriptional transactivator (rtTA) mice (obtained via Mutant Mouse Regional Resource Centers (MMRRC)) (32) to generate double-transgenic mice. In addition to the set of PCR primers above, mice carrying both transgenes were selected using the following rtTA PCR primers: forward rtTA, GTG ATT AAC AGC GCA CTG GAG; reverse rtTA, CAA ACA GTT CGA TAG CTT GCC G. Additionally, founder lines were selected on the basis of their lack of transgenic \(\alpha_{1C}\) expression in the absence of doxycycline. Mice were fed food impregnated with 0.2 g/kg doxycycline to induce expression (Bio Serv, catalog no. S3888) for 1–2 days. The Institutional Animal Care and Use Committee at Columbia University approved all animal experiments.

**Immunoblots and Immunofluorescence**—Cardiomyocytes were isolated (33) from 8- to 12-week-old non-transgenic and doxycycline-fed transgenic mice. Cardiomyocytes were homogenized in a 1% Triton X-100 buffer containing 50 mM Tris-HCl (pH 7.4) 150 mM NaCl, 10 mM EDTA, 10 mM EGTA, and protease inhibitors. The lysates were incubated on ice for 30 min, centrifuged at 14,000 rpm at 4 °C for 10 min, and supernatants were collected. Proteins were size-fractionated on SDS-PAGE, transferred to nitrocellulose membranes, and probed with HRP-conjugated antibody and anti-\(\alpha_{1C}\) and HRP-conjugated goat anti-rabbit antibodies. Detection was performed with a charge-coupled device camera (Carestream Imaging). Loading normalization was performed with anti-tubulin antibody (Santa Cruz Biotechnology). For immunofluorescence, isolated cardiomyocytes were fixed for 15 min in 4% paraformaldehyde. Indirect immunofluorescence was performed using a 1:200 FITC-labeled goat anti-rabbit antibody (Sigma) and 1:200 HRP-conjugated antibody (Sigma). Images were acquired using a confocal microscope.

**Cellular Electrophysiology**—The isolated cardiomyocytes were superfused with 140 mM tetraethylammonium-Cl, 1.8 mM CaCl\(_2\), 1 mM MgCl\(_2\), 10 mM glucose, and 10 mM HEPES, adjusted to pH 7.4 with CsOH. All experiments were performed at room temperature, ~22 °C. Membrane currents were measured by whole-cell patch clamp method using a MultiClamp 700B amplifier (Axon Instruments). The pipette solution contained (in mM) 135 mM CsCl, 10 mM EGTA, 1 mM MgCl\(_2\), 2 mM magnesium-ATP, 2.0 mM CaCl\(_2\), and 10 mM HEPES, adjusted to pH 7.2 with CsOH. Pipette series resistances were usually <1 M\(\Omega\) after 60% compensation. Leak currents and capacitance transients were subtracted by a P/4 protocol. To measure Ca\(^{2+}\) peak currents, the cell membrane potential was held at −70 mV and stepped to +10 mV for 350 ms every 10 s. To evaluate the current–voltage relationship for Ca\(^{2+}\) currents, the same protocol was repeated with steps between −50 mV to +50 mV in 10-mV increments.

**Fractional Shortening**—Freshly isolated myocytes were perfused with Tyrode solution containing 1.8 mM CaCl\(_2\). Myocytes were field-stimulated at 1 Hz. Nisoldipine (300 nM) dissolved in Tyrode solution was then superperfused. Fractional shortening of sarcomere length was measured using the SarcLen module of Ionoptix.
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RESULTS

Generation of Inducible, Cardiac-specific ΔPDZ α1C Transgenic Mice—A class I PDZ domain-binding motif VSXL is present in the cardiac/neuronal α1C subunit, conforming to the consensus sequence X[T/S]XϕC_OH where X is any residue and ϕ is a hydrophobe (34). The PDZ domain-binding motif is conserved across many species, including human, rabbit, rodents, and zebrafish and in the Caenorhabditis elegans I-type Ca2+ channel subunit (egl-19) (23) (Fig. 1). The role for the PDZ ligand domain in the heart is unknown. Cultured neurons transfected, using a Ca2+ phosphate technique, with α1C lacking the PDZ ligand domain exhibited attenuated cAMP-response element-binding protein responses, indicating an important role for the PDZ ligand motif in the subcellular localization of α1C in neurons (21). Although adenoviruses have been used to express Ca,1.2 subunits in cardiomyocytes (35, 36), creation of adenoviruses encoding α1C is difficult because of the α1C insert size and because viral infection requires that cardiomyocytes be cultured for extended periods. Although useful for investigating biophysical properties, many relevant aspects of Ca1.2 channel targeting and functional modulation in heart cannot be studied in heterologous expression systems, which lack the complex cytoarchitecture and intracellular environment of adult cardiomyocytes.

To circumvent these problems, we generated transgenic mice featuring inducible, cardiac-specific expression of DHP-resistant, FLAG epitope-tagged α1C (Fig. 2A). This approach preserves the hormonal regulation of Ca1.2 by limiting α1C overexpression, is relatively rapid, and enables functional screening of α1C mutants in cardiomyocytes freshly isolated from adult mice. We previously generated transgenic mice with inducible expression of DHP-resistant (T1066Y/Q1070M), N-terminal, 3X FLAG epitope-tagged α1C, designated pWT α1C (37). These transgenic mice were crossed with α-MHC rtTA transgenic mice. To determine the importance of the PDZ domain-binding site in α1C in cardiomyocytes, we generated a transgenic mouse line expressing α1C with a deletion of the four C-terminal amino acid residues in the background of an N-terminal 3X FLAG epitope tag and DHP resistance (ΔPDZ). These transgenic mice were crossed with α-MHC-rtTA, and double-transgenic mice were identified by PCR (Fig. 2B). Several ΔPDZ-α1C founder transgenic lines were originally generated, and all lines demonstrated doxycycline-induced α1C expression after crossing with the αMHC-rtTA mice. The results were consistent across all founder lines and gender, and therefore were pooled.

In cardiomyocytes isolated from non-transgenic mice (C57Bl/6), native α1C was detected as a full-length, ~240 kDa band and a cleaved, ~210 kDa band using an anti-α1C antibody created against an internal epitope within the intracellular loop of domains II and III. Native α1C in non-transgenic mice cannot be detected using an anti-FLAG antibody (Fig. 2C). Both the pWT α1C transgenic channels and the transgenic channels were detected using anti-FLAG antibody. The ratios of cleaved to full-length pWT and ΔPDZ transgenic α1C were not significantly different, implying that the PDZ motif is not required for cleavage of the α1C distal C terminus.

Confirming the expression of the transgene, immunofluorescence staining of fixed cardiomyocytes from pWT and ΔPDZ mutant transgenic mice with an anti-FLAG antibody showed a membrane distribution of expressed α1C subunits consistent with t-tubular localization (Fig. 2D). No staining was detected in cardiomyocytes when the anti-FLAG antibody was omitted.

The PDZ Motif Is Not Required for Trafficking to the Dyad or Initiating E-C Coupling—Cardiomyocyte contraction requires Ca2+ influx via Ca1.2, which triggers sarcoplasmic reticulum Ca2+ release. We assessed the localization of the transgenic channels in the dyad by determining whether E-C coupling could be maintained in the presence of nisoldipine. In control non-transgenic cardiomyocytes, 300 nM nisoldipine eliminated contraction to electric field stimulation at 1 Hz (Fig.
3, A and D). By contrast, in cardiomyocytes from both the pWT-α1C and the ΔPDZ-α1C transgenic mice, the effect of nisoldipine was greatly diminished, and E-C coupling was preserved (Fig. 3, B–D). Taken together, these results demonstrate that the PDZ ligand motif is not required for the surface expression and subcellular localization of CaV1.2 to the dyad. Furthermore, the PDZ ligand motif is not required for the initiation of E-C coupling in mice.

Functional, Inducible Expression of ΔPDZ-α1C in Cardiomyocytes—To measure transgenic channel selectivity, we chose a concentration of 300 nM nisoldipine as optimal because nisoldipine (300 nM) blocked 98% of heterologously expressed WT CaV1.2 current in tsA-201 cells but only blocked 34.6% of DHP-insensitive α1C (37). We measured CaV1.2 currents in adult cardiomyocytes from non-transgenic and transgenic mice. Nisoldipine (300 nM) inhibited 92.4% ± 1.6% of endogenous peak Ca2+ current in cardiomyocytes isolated from non-transgenic mice (n = 12) (Fig. 4, A and F) but 63.4% ± 4.7% of peak current in cardiomyocytes isolated from doxycycline-fed pWT-α1C transgenic mice (n = 30) (Fig. 4, B, D, and F) and 64.4% ± 2.8% of peak current in cardiomyocytes isolated from doxycycline-fed ΔPDZ mutant transgenic mice (n = 25 cardiomyocytes, p ≤ 0.001) (Fig. 4, C, E, and F). The voltage dependence of CaV1.2 activation for ΔPDZ α1C was not different from the endogenous channels because the current-voltage curves in the absence and presence of nisoldipine were identical. These findings imply that, at least under basal conditions, the modulation of the transgenic, PDZ-deleted CaV1.2 channels by accessory proteins was similar to endogenous CaV1.2 channels.

Role of PDZ Motif in the Adrenergic Modulation of CaV1.2—The PDZ-dependent association of α1C with Cypher/ZASP, functioning as an protein kinase A anchoring protein (AKAP) has been proposed recently to facilitate β-adrenergic-mediated phosphorylation of CaV1.2. Deletion of the α1C C-terminal PDZ motif, preventing association of CaV1.2 and Cypher/
ZASP, significantly impaired isoproterenol-stimulated PKA phosphorylation of Ser1928 of heterologously expressed CaV1.2 (26). The role of the PDZ ligand-binding motif in regulating the functional modulation of CaV1.2 by the adrenergic system was not assessed.

Freshly isolated cardiomyocytes were isolated from doxycycline-treated /H9004 PDZ transgenic mice. In the presence of nisoldipine, isoproterenol increased peak CaV1.2 current by a mean of 1.8 ± 0.1-fold, identical to the isoproterenol-induced augmentation of current in pWT1C transgenic cardiomyocytes (p not significant, pWT1C versus ΔPDZ) (Fig. 5, A–C and F). In the presence of nisoldipine, forskolin increased peak CaV1.2 current by a mean of 1.7 ± 0.04-fold increase in cardiomyocytes isolated from the ΔPDZ mice, nearly identical to the 1.8 ± 0.1-fold increase in pWT1C cardiomyocytes (Fig. 5, D–F). Taken together, these results demonstrate that the CaV1.2 PDZ ligand motif is not required for surface expression, dyadic localization, E-C coupling, or adrenergic modulation of Ca²⁺ currents in cardiomyocytes.

DISCUSSION

The current understanding regarding mechanisms underlying CaV1.2 trafficking and modulation derives from studies of recombinant channels reconstituted in heterologous cells. An important limitation is that heterologous cells lack the complex cytoarchitecture and intracellular milieu of adult cardiomyocytes. To address this, we developed an approach that utilizes transgenic mouse expressing doxycycline-inducible, cardiac-specific, DHP-resistant α1C. Importantly, the transgenic wild-type channels are transported appropriately to the dyad, can initiate E-C coupling, demonstrate normal activation and inactivation properties, and are fully and appropriately regulated by β-adrenergic stimulation. Compared with knockin mouse models, this approach is both cost-effective and rapid and, perhaps more importantly, enables the induced brief expression of mutant channels in adults, permitting the comparison of WT and mutant α1C structure-function mechanisms in the absence of developmental abnormalities and heart failure. This is not possible in knockin mouse models, which display embryonic lethality (such as α1C1904 and α1C1796 knockin mice) (17, 18).

Importantly, this approach avoids the need to culture cardiomyocytes, which may cause dedifferentiation, and uses an inducible expression system so that we can carefully titrate the level of expression.

The most important potential limitation of this approach is that the transgenic Ca²⁺ channels may compete with endogenous channels for limited binding partners, therefore altering...
the stoichiometry of the channels. Our experiments were designed to limit overexpression by exposing the animals to doxycycline for only 1–2 days and selecting for transgenic founder lines with relatively low expression. The findings that the trafficking and function of the pWT \( \text{H9251}^{1C} \) transgenic channels were normal suggest that limited overexpression does not adversely affect the normal characteristics of the channels. The trafficking and function of the \( \Delta \text{PDZ} \) mutant channels were similar to both endogenous and pWT \( \text{H9251}^{1C} \) channels, demonstrating that the PDZ motif of \( \text{H9251}^{1C} \) is not required in adult cardiomyocytes.

The distal C terminus of the \( \text{H9251}^{1C} \) subunit regulates \( \text{CaV}1.2 \) trafficking and function in the heart, but the mechanisms and determinants are unknown. The deficits in \( \text{CaV}1.2 \) trafficking and \( \beta \)-adrenergic regulation seen in \( \alpha_{1C}^{1904} \) (17) and \( \alpha_{1C}^{1796} \) knockin mice could be due to multiple factors, including deficits in macromolecular complex formation (17, 18). In vitro binding assays demonstrated that the \( \alpha_{1C} \) PDZ ligand motif was required for interactions between \( \text{CaV}1.2 \) and several PDZ domain proteins expressed in cardiomyocytes, such as NHERF1, MAGI-3, and nNOS (27). This sequence, or a nearly identical sequence, is present in human, monkey, mouse, rat, marmoset, rabbit, and zebrafish \( \alpha_{1C} \). On the basis of the postulated role of the PDZ motif of \( \alpha_{1C} \) in regulating cAMP-response element-binding protein signaling in neurons, potentially via its effects on subcellular localization (21) and adrenergically mediated phosphorylation of Ser\(^{1928} \) of \( \text{CaV}1.2 \) in neonatal cardiomyocytes via Cypher/ZASP (26), we hypothesized that disrupting PDZ-mediated interactions would affect \( \text{CaV}1.2 \) trafficking and function in cardiomyocytes. \( \alpha_{1C} \) Ser\(^{1928} \), which was originally identified as the sole \( \alpha_{1C} \) PKA phosphorylation site (9, 38–45), is not required for \( \beta \)-adrenergic receptor stimulation of \( \text{CaV}1.2 \), as shown in cardiomyocytes infected with adenovirus expressing a DHP-resistant S1928A-\( \alpha_{1C} \) (35) and in an \( \alpha_{1C} \) S1928A knockin mouse (46). The role for Ca\(^{2+} \) channels in mediating cAMP-response element-binding protein signaling in the heart has not been delineated.

PDZ-domain proteins are important modulators of K\(^{+} \) (47, 48) and Na\(^{+} \) channel (49–51) function and \( \beta \)-adrenergic signaling (52) in the heart. PDZ domain interactions are regulated

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**FIGURE 4.** Dihydropyridine-resistant currents in \( \Delta \text{PDZ} \) transgenic mice. **A–C**, examples of whole-cell \( \text{CaV}1.2 \) currents recorded from pulses from \(-70 \text{ mV to } +10 \text{ mV} \) before (black traces) and 3 min after (red traces) 300 nM nisoldipine. \( \text{NTG} \), non-transgenic. **D and E**, \( \text{CaV}^{1.2} \) current (I\(_{\text{Ca}} \))-voltage relationships of pWT (D) and \( \Delta \text{PDZ} \) (E) \( \text{CaV}^{1.2} \) acquired before (black trace) and 3 min after superfusion of 300 nM nisoldipine. Insets, series of whole-cell \( \text{CaV}1.2 \) currents recorded from a series of pulses between \(-40 \text{ mV to } +60 \text{ mV} \) from a holding potential of \(-70 \text{ mV} \) in the absence of nisoldipine (black traces) and 3 min after 300 nM nisoldipine (red traces). pF, picofarad. F, fraction of DHP-resistant current density. Data are mean ± S.E. ***, \( p < 0.001 \) by Student’s t test, analysis of variance, and Sidak post hoc test.
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by phosphorylation and facilitate intracomplex phosphorylation. The adaptor SAP97 facilitates the phosphorylation of Kv4.2 and Kv4.3 by Ca\(^{2+}\)-calmodulin kinase II (47) and of the β1-adrenergic receptor by PKA (53). In the heart, both Na\(_v\)1.5 and Kir2.1 interact independently with PDZ domain proteins, regulating the surface expression and function of the channels (54–56). The expression of Na\(_v\)1.5 also affects the turnover and regulation of Kir2.1 channels in the heart via PDZ-dependent interactions (55). Mice with deletion of the Na\(_v\)1.5 PDZ motif displayed reduced Na\(_v\)1.5 expression and current, specifically at the lateral myocyte membrane, whereas expression and function at the intercalated disks were not altered (51). Ca\(_\text{v}\)1.3, which is expressed in neurons and in cardiac sinoatrial and atrioventricular nodes and atria, but not in ventricles (57), also contains a C-terminal PDZ ligand motif and binds erbin (58) and shank, both neuronal PDZ domain proteins (23, 59).

The most direct approach to determine the functional role of the PDZ domain proteins in modulating Ca\(_\text{v}\)1.2 is to express a PDZ-deleted α1C in cardiomyocytes. This approach avoids potential nonspecific effects of deleting the PDZ-binding domain proteins instead because they also interact with other ion channels and β-adrenergic receptors. Using this approach, we found that deletion of the α1C PDZ ligand motif does not alter Ca\(_\text{v}\)1.2 trafficking, basal function, or modulation by the sympathetic nervous system in adult cardiomyocytes. We cannot exclude the possibility that the α1C PDZ ligand motif may be responsible for functions during cardiac development or may have a role in other tissues. An advantage of our approach is that the short-term induction of expression minimizes the compensatory effects that can be observed in more traditional transgenic expression or knockin mice.

Taken together, we show that the α1C PDZ ligand motif is not required for trafficking to the surface or dyads, basal function, and adrenergic modulation in the heart. The mechanisms responsible for how the distal C terminus regulates Ca\(_\text{v}\)1.2 trafficking and function in cardiomyocytes have yet to be discovered.

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