Homodimerization of the Src Homology 3 Domain of the Calcium Channel β-Subunit Drives Dynamin-dependent Endocytosis

Erick Miranda-Laferte, Giovanni Gonzalez-Gutierrez, Silke Schmidt, Andre Zeug, Evgeni G. Ponimaskin, Alan Neely, and Patricia Hidalgo

Voltage-dependent calcium channels constitute the main entry pathway for calcium into excitable cells. They are heteromultimers formed by an α1 pore-forming subunit (CaVα1) and accessory subunits. To achieve a precise coordination of calcium signals, the expression and activity of these channels is tightly controlled. The accessory β-subunit (CaVβ1), a membrane associated guanylate kinase containing one guanylate kinase (β-GK) and one Src homology 3 (β-SH3) domain, has antagonistic effects on calcium currents by regulating different aspects of channel function. Although β-GK binds to a conserved site within the α1 pore-forming subunit and facilitates channel opening, β-SH3 binds to dynamin and promotes endocytosis. Here, we investigated the molecular switch underlying the functional duality of this modular protein. We show that β-SH3 homodimerizes through a single disulfide bond. Substitution of the only cysteine residue abolishes dimerization and impairs internalization of L-type CaVα1.2 channels expressed in Xenopus oocytes while preserving dynamin binding. Covalent linkage of the β-SH3 dimerization-deficient mutant yields a concatamer that binds to dynamin and restores endocytosis. Moreover, using FRET analysis, we show in living cells that CaVαβ form oligomers and that this interaction is reduced by CaVα1 β, Association of CaVβ with a polypeptide encoding the binding motif in CaVα1 inhibited endocytosis. Together, these findings reveal that β-SH3 dimerization is crucial for endocytosis and suggest that channel activation and internalization are two mutually exclusive functions of CaVαβ. We propose that a change in the oligomeric state of CaVαβ is the functional switch between channel activator and channel internalizer.

Voltage-dependent calcium channels link membrane depolarization to transient increases in cytosolic calcium concentra-

© 2011 by The American Society for Biochemistry and Molecular Biology, Inc. Printed in the U.S.A.
the β-SH3 domain does bind to dynamin (7), suggesting that the PxPxP binding motif becomes exposed by a yet to be discovered regulatory event. Furthermore, following association of the SH3 domain of Ca₃β with dynamin, the number of channels at the plasma membrane is reduced (7). Conversely, association of the GK domain of Ca₃β with a highly conserved binding motif in Ca₃α₁, known as the α₁-interaction domain (AID) (17) favors the coupling between voltage and channel protein. GST alone and GST fused to the intracellular loop constructs bear a His₆ tag fused at the N-terminal end of the protein. Dynamic proteins were eluted with SDS-PAGE loading buffer and resolved on 4–25% polyacrylamide gel (4–25%) overnight at 4 °C using cathode buffer containing 100 mM histidine, 0.002% Serva Blue G and 0.05% SDS, or both. Proteins were resolved in a gradient polyacrylamide gel (4–25%) overnight at 4 °C using cathode buffer containing 100 mM histidine, 0.002% Serva Blue G powder (SERVA electrophoresis), pH 8.0, and anode buffer containing 100 mM Tris, pH 8.8.

EXPERIMENTAL PROCEDURES

**cDNA Constructs and Recombinant Proteins**—The Ca₃.1.2 channel construct and dynamin has been described elsewhere (7). Full-length Ca₃β₂a (Swiss-Prot entry Q8VGC3), β-SH3, and β-SH3 C113A monomer and concatamer versions were prepared as described (7, 25). All recombinant protein constructs bear a His₆ tag fused at the N-terminal end of the protein. GST alone and GST fused to the intracellular loop encompassing the AID site from Ca₃.2.3, hereby referred to as GST-AID, were produced as described previously (25).

**Pulldown Assays**—Pulldown assays with His-tagged β-SH3 derivatives were performed as described (7). Cobalt-based agarose beads were coupled to the proteins and incubated with pre-cleared extract from tsA201 cells transfected with a dynamin-encoding plasmid 24 to 36 h earlier. Bound proteins were eluted with SDS-PAGE loading buffer and resolved on SDS-PAGE. Immunoblot analysis was done using anti-dynamin antibody (BD Biosciences).

**Xenopus Oocyte Preparation, Injection, and Electrophysiological Recordings**—Xenopus laevis oocytes were prepared, injected, and maintained as in a previous report (26). Electrophysiological recordings on Ca₃.1.2 expressing oocytes were performed using the cut-open oocyte technique with a CA-1B amplifier (Dagan Corp., Minneapolis MN) as described (25). Gating currents were separated from ionic currents by stepping the membrane voltage near the reversal potential for the permeant ion (Ba²⁺) (7). Data acquisition and analysis were performed using the pCLAMP system and software (Axon Instruments Inc., Foster City, CA). Currents were filtered at 2 kHz and digitized at 10 kHz. Linear components were eliminated by P/4 prepulse protocol that consisted of four consecutives pulses a fourth of the amplitude of the test pulse. Current traces during these prepulses were added together and subtracted from the current traces obtained with the main pulse.

FRET Analysis—Ca₃β₂ was fused at its carboxyl-terminal end to either YFP (Ca₃β-YFP) or CFP (Ca₃β-CFP) by standard PCR methods. Mammalian tsA201 cells grown on coverslips (15-mm diameter) were co-transfected with YFP- and CFP-tagged Ca₃β₂-encoding plasmids using Lipofectamine™ (Invitrogen). Spectral analysis of living tsA210 cells co-expressing CFP- and YFP-tagged Ca₃β₂ constructs were performed according to Kobe et al. (27). Quantitative linear unmixing FRET (lux-FRET) analysis in cuvettes and at the single cell level were performed 18–24 h after transfection as described recently (28, 29). Lux-FRET allows determination of FRET efficiencies of donor-acceptor pairs in the presence of free donor and acceptor molecules.

**RESULTS**

β-SH3 Dimerizes through a Single Disulfide Bond—Here, we studied the quaternary structure of β-SH3 by BN-PAGE that is particularly suited to analyze the oligomeric structure of proteins (31–34). The first hint that β-SH3 forms dimers came from size-exclusion chromatography of isolated β-SH3 indicating some degree of dimerization (35) and from non-reducing SDS-denaturing PAGE showing a small fraction resistant to denaturation (supplemental Fig. 1). In BN-PAGE, β-SH3 migrates in two bands, suggesting the co-existence of two oligomeric states (Fig. 1B). Incubation with SDS resulted in no significant changes in the mobility or relative intensity of both bands. In contrast, treatment with the reducing agent DTT causes the disappearance of the upper band, suggesting that this oligomer is generated by disulfide bonding. Because the mobility of the protein in non-denaturing electrophoresis depends on the shape, the numbers of monomers assembled per protein complex cannot be readily determined. To address this issue, we engineered a concatenated cDNA construct by linking two β-SH3 coding regions in a single open reading frame to yield a concatameric β-SH3 protein. This concatamer eluted from a size-exclusion column slightly earlier than its monomeric counterpart and was resolved at the expected molecular mass by reducing SDS-PAGE (Fig. 1C). In BN-PAGE, the upper band of the non-covalently linked β-SH3 migrated at the same position as the concatamer, demonstrating that it corresponds to a dimeric form and, accordingly, the lower molecular band to the monomeric form.

To further assess the contribution of disulfide bonding to dimer formation, we substituted the single cysteine residue in β-SH3, located at position 113, by alanine (β-SH3 C113A) and followed the same expression and purification methodology developed for wild-type β-SH3 (7). β-SH3 C113A elutes as a monodisperse peak from a size-exclusion column at the same position as wild-type β-SH3 (Fig. 1C), but in BN-PAGE, it migrates as a single band faster than the β-SH3 concatamer.
Dimerization of SH3 Domain Mediates β-subunit Endocytosis

FIGURE 1. Wild-type β-SH3 domain but not β-SH3 C113A forms dimers. A, schematic and ribbon representation of Caβ₂ structure in complex with the highly conserved β-binding site of Caα₁ (AID, helix shown in gray) (Protein Data Bank code 1TOJ). SH3, shown in gray, and GK, shown in black, modulate different channel functions. The two N-terminal cysteine residues that undergo palmitoylation in Caβ₂, variant used in this study are marked by zigzag lines in the sketch at the top of the panel. B, oligomeric state of wild-type β-SH3 determined by BN-PAGE. Lanes 1–4 were loaded with β-SH3, and lanes 5 and 6 were loaded with β-SH3 concatamer. To induce dissociation into lower-order oligomeric states, including monomers, samples were treated as indicated at the bottom of the panel as described under “Experimental Procedures.” C, size-exclusion chromatography profile (Superdex 200 10/30 column, GE Healthcare) of β-SH3 C113A and β-SH3 concatamer. The corresponding profile for wild-type β-SH3 has been shown elsewhere (7), and its peak, corresponding to the monomeric fraction, is denoted here with an open arrowhead. The elution volume of molecular weight standards are indicated above the chromatogram. Numbers correspond to molecular masses in kDa. Vo, void, 67.0 (albumin); 43.0 (ovalbumin); and 13.7 (ribonuclease). The adjacent panel shows the same proteins resolved onto a reducing SDS-PAGE: lane 1, molecular weight standards; lane 2, wild-type β-SH3; lane 3, β-SH3 C113A; and lane 4, β-SH3 concatamer. D, oligomeric state of β-SH3 C113A determined by BN-PAGE. Lanes 1–3 were loaded with β-SH3 C113A, and lane 4 was loaded with β-SH3 concatamer. Samples were treated as indicated.

(Fig. 1D). This migration pattern was not altered by treatment with either SDS or DTT demonstrating that β-SH3 C113A does not form dimers. We conclude that wild-type β-SH3 forms homodimers bound covalently through a single disulfide bridge involving cysteine residue 113.

β-SH3 C113A Dimerization-deficient Mutant Associates with Dynamin but Does Not Promote Endocytosis—We next examined whether the dimeric quaternary structure of β-SH3 plays a role in regulating endocytosis. In a standard pulldown assay, His-tagged wild-type and C113A β-SH3 used as bait brought down dynamin expressed in tsA201 cells (Fig. 2A).

Thus, eliminating dimer-forming cysteine 113 did not prevent association with dynamin but, as revealed by charge-movement assay, it abolished the capability of wild-type β-SH3 or β-SH3 C113A to associate with dynamin but does not internalize channels predicts that bring-
Dimerization of SH3 Domain Mediates β-subunit Endocytosis

FIGURE 3. Covalently linking two molecules of β-SH3 C113A dimerization-deficient mutant restores the endocytic capability. A, Western blot (WB) of a pulldown assay was done as described in the legend to Fig. 2 but instead used concatameric versions of β-SH3 and β-SH3 C113A. Lysate from cells expressing dynamin were incubated with either β-SH3 concatamer (lane 1) or β-SH3 C113A concatamer (lane 2) or with cobalt beads alone (lane 3). B, CMBl assay results from Xenopus oocytes expressing CaV1.2 alone and in combination with β-SH3 either as a concatamer or non-covalently linked. The bar graph shows N × q values from oocytes expressing the indicated channel-protein combinations; CaV1.2 alone (145), CaV1.2 alone and in combination with either β-SH3 concatamer (146) or β-SH3 C113A concatamer (147) or with cobalt beads alone (148). Average N × q values were significantly smaller in the presence of β-SH3 (p < 5.7 × 10⁻⁴) or β-SH3 concatamer (p < 2.4 × 10⁻⁴) compared with CaV1.2 alone. Values for N × q between monomer and concatamers were also different but to a smaller degree (p < 0.04), C, as same as B, but from Xenopus oocytes expressing CaV1.2 alone and in combination with either a β-SH3 C113A concatamer or β-SH3 C113A. The bar graph shows average N × q values for the indicated channel-protein combinations; CaV1.2 alone (149), CaV1.2 + β-SH3 C113A (150), or CaV1.2 + β-SH3 C113A (151). Average N × q values were significantly smaller for CaV1.2 + β-SH3 C113A concatamer than for CaV1.2 alone (p < 4.2 × 10⁻⁴) or CaV1.2 + β-SH3 C113A (p < 2.9 × 10⁻³) according to a two-tailed t test. 

We engineered a concatamer β-SH3 C113A and compared it with the wild-type β-SH3 concatamer. Both concatamers eluted from a size-exclusion column at the same position (supplemental Fig. 2), and both retained the ability to associate with dynamin (Fig. 3A). The dimerization-competent molecules joined in a single polypeptide chain also retained the endocytic phenotype of the non-covalently linked β-SH3 (Fig. 3B), indicating that the molecular linkage did not introduce major structural rearrangements. More remarkably, linking two molecules of the dimerization-deficient mutant indeed restored β-SH3 ability to internalize CaV1.2 channels (Fig. 3C). This result highlights the functional relevance of β-SH3 dimerization for endocytosis.

Membrane-targeted Caβ2a Isoform Forms Dimers—We have shown previously that when association with Caβα1 is prevented, Caβ2a is as potent as the SH3 domain alone in downregulating HVA channels (7). Caβ2a is a unique variant of Caβ that undergoes dynamic palmitoylation (see Fig. 1A), and it is targeted to the plasma membrane even in the absence of the Caβα1 pore-forming subunit (38, 39). To investigate intermolecular interactions within Caββ, we examined FRET occurrence between fluorophore-labeled Caβ2a in living cells. Fluorophores CFP and YFP were fused to the C terminus to yield Caβ2a-CFP and Caβ2a-YFP, respectively. Fig. 4A shows the fluorescence emission spectra measured in a cuvette containing cells transfected with Caββ2a-CFP and Caββ2a-YFP encoding plasmid together or separately. Only cells coexpressing Caββ2a-CFP and Caββ2a-YFP demonstrated a larger emission peak at 525 nm concomitant with a smaller CFP emission that reflects the energy transferred from the donor CFP to the acceptor YFP. These results indicate that Caββ2a oligomerizes within living cells. To calculate the apparent FRET efficiency (EFP) we applied the lux-FRET approach (28) and found that EFP was 10 ± 1% in co-transfected cells, whereas this value was 0 ± 1% in the mix of single transfected cells. Combination of FRET analysis with confocal microscopy revealed that Caββ2a-CFP and Caββ2a-YFP are localized almost entirely at the plasma membrane and that membrane-anchored Caββ2a showed the highest EFP (10.8 ± 1.5%), whereas FRET value measured in minor cytosolic fraction of Caββ2a (1.7 ± 0.4%) was close to that obtained for negative control (0 ± 0.4%) (Fig. 4B and supplemental Movie). Fusing the fluorescence proteins to the N-terminal end of Caββ2a drastically change its intracellular localization from membrane-bound to homogenously distributed throughout the entire cell.

YFP and CFP proteins linked together in a single polypeptide chain (tandem construct) yields an EFP of ~35% when measured under the same experimental conditions. This value sets an upper limit of 17.5% to EFP in co-transfected cells as only 50% of the β-subunit dimers carry the YFP-CFP pair, the remaining fraction composed of YFP-YFP and CFP-CFP complexes are not detectable by FRET. Thus, the EFP of 10% obtained in the present study indicates that in intact cells an important fraction of Caββ2a oligomerizes.

In BN-PAGE, Caββ2a display a migration pattern consistent with dimer formation as it compares to the one obtained with a concatenated Caββ protein construct (Fig. 4C). The Caββ2a concatamer shows an additional band that corresponds to monomeric Caββ likely produced by proteolysis (supplemental Fig. 3) (40). In contrast to β-SH3, Caββ2a dimers were resistant to incubation with either SDS or DTT and could only be disrupted by treatment with both agents (Fig. 4C). Substitution of C113 by alanine (Caββ2a C113A) did not prevent dimer formation (Fig. 4D), indicating that other regions of the full-length protein contribute to the dimerization interface. This idea is supported by our earlier experiments demonstrating that isolated GK domain forms dimers (18). In addition, a recent report showed higher-order oligomerization in cytosolic Caββ variants coexpressed with the Caβα1 pore-forming subunit that depends on specific sites within the GK domain (41). From our experiments, the existence of less stable higher organized oligomeric forms of Caββ2a cannot be excluded.

Preassociation of Caββ2a with a Polypeptide Encoding Binding Motif of α1, Pore-forming AID Inhibits Endocytosis—We have shown previously that full-length Caββ2a is as efficient as the SH3 domain to promote endocytosis of Caβα1 when association to it is prevented and that it also induces internalization of the distantly related Shaker potassium channel (7). Deletion of the highly conserved AID sequence located in the intracellular loop joining the first and second domain of Caβ1.2 (Caβ1.2 ΔAID) effectively disrupts any Caββ-Caβα1 association. Here, we used the same strategy to follow endocytosis mediated by Caββ2a. Caβ1.2 ΔAID channels were internalized by Caββ2a...
wild-type as well as by the C113A mutant. This result emphasizes that dimer formation rather than the chemical nature of the residue at this position is critical for endocytosis (Fig. 5A). The fact that CaV1.2a efficiently internalizes channels lacking the AID site suggests that its association with the pore-forming subunit hinders channel internalization. To test this idea, we preincubated CaV1.2a with GST-AID, a GST-fusion protein that included the AID sequence, and performed CMBI assay. Association of CaV1.2a with the AID site inhibited endocytosis (Fig. 5B). These findings are consistent with a model whereby channel activation and internalization are mutually exclusive functions of CaV1.2a.

**DISCUSSION**

It is increasingly evident that SH3-containing proteins expand their functional diversity by dimerization. For example, in islet brain 1 protein, mutations that destabilize dimer formation do not affect binding with the proline-rich region containing partner c-Jun N-terminal kinase but impairs its ability to regulate insulin secretion (23). In the case of CT10 regulator of kinase-like adaptor protein, a functionally relevant nuclear export signal becomes available only after a conformational change induced by homodimerization (24). Dimerization of CaV1.2a-SH3 could position two dynamin molecules together promoting its oligomerization and GTPase activity (42) or could bring dynamin close to other proteins of the endocytic machinery, serving as a local adaptor that recruits an active endocytic complex. This idea is supported by the observation that two CaV1.2a-SH3 modules brought together by either a disulfide bond or by a covalently attached peptide linker are equally competent for activation of endocytosis. Thus, closeness of the two SH3 domains appears sufficient for function as opposed to the

---

**FIGURE 4.** Membrane-targeted CaV1.2a also forms dimers. A, fluorescence emission spectra of living tsA210 cells expressing the indicated CFP- and YFP-tagged CaV1.2a constructs either separately or together (co-transfection). Mix corresponds to the emission spectra of a mixture of single transfected cells (mix). Emission spectra were collected at excitation wavelength $\lambda_{ex} = 440$ nm. a.u., arbitrary units. B, distribution and quantitative pixel-based FRET analysis of CFP- and YFP-tagged CaV1.2a in living tsA210 cells. Scale bar, 5 $\mu$m. Ef, apparent FRET efficiency, mem, membrane-localized CaV1.2a; intra, intracellular-localized CaV1.2a. Data represent mean values ± S.E. (n = 6). C, BN-PAGE analysis of CaV1.2a. Lanes 1–4 were loaded with CaV1.2a and lanes 5 and 6 with the CaV1.2a concatamer. In the absence of SDS, the CaV1.2a concatamer is poorly resolved (lane 5). Samples were treated as indicated at the bottom of the panel. D, BN-PAGE analysis of CaV1.2a C113A. Lanes 1–4 were loaded with CaV1.2a C113A and lanes 5 and 6 were loaded with the CaV1.2a concatamer.
Dimerization of SH3 Domain Mediates β-subunit Endocytosis

A

CaV1.2 ΔAID

B

CaV1.2 ΔAID

C

CaV1.2 ΔAID

N × q [pC]

N × q [pC]

N × q [pC]

0

100

200

0

100

200

0

100

200

Caβ2α Caβ2α

Caβ2α C113A

Caβ2α + GST Caβ2α + GST-AID

Caβ2α

Caβ2α

Caβ2α

Caβ2α

CaV1.2

CaV1.2

CaV1.2

FIGURE 5. Association of CaVβ2α with the AID motif abolishes channel internalization and impairs dimerization. A, bar graph showing average N × q values for the indicated channel-protein combinations. CaV1.2 ΔAID alone (200 ± 29 pC, n = 14) was significantly larger than following injection of either CaVβ2α wild-type (40 ± 11 pC, n = 8) or C113A mutant (58 ± 9 pC, n = 18). B, bar graph showing average N × q values for the following: CaV1.2 ΔAID alone (119 ± 13 pC, n = 16) and after injection of CaVβ2α either preincubated with GST (23 ± 4 pC, n = 11) or with GST-AID (104 ± 15 pC, n = 17). C, FRET efficiencies for CaVβ2α-CFP and CaVβ2α-YFP proteins coexpressed in the absence (7.6 ± 0.3%) and presence (3.7 ± 0.3%) of α1 pore-forming subunit. FRET experiments were repeated three times with consistent results.

requirement of a precise geometry of the two dynamin binding sites within the quaternary structure of the dimer. Possible candidates to be recruited are members of the Ras superfamily of monomeric small GTPases known to inhibit CaV1.2-mediated currents by interacting with CaVβ subunits (43). Furthermore, the effect of the Ras protein REM involves dynamin-dependent endocytosis (44), suggesting that REM and dynamin may simultaneously interact with the CaVβ dimer to inhibit calcium currents. The repertoire of regulatory inputs to the endocytic machinery may be augmented even further in full-length CaVβ by the GK domain, which also appears as a protein-protein interaction domain (45–47). Interaction of kinesin-like proteins with GK domains (48) suggests that CaVβ may be engaged in the sorting of the endocytic vesicle by motor proteins. We have already proposed that the functional duality of CaVβ constitutes an efficient quality control mechanism in which the same protein ensures functional fitness and survival of the channel in the plasma membrane (7). In this context, it appears natural to think that channels internalized by CaVβ are directed to early endosomes for recycling rather than to lysosomes for degradation.

Our results hint to a mutual exclusion between channel activation and internalization by CaVβ. Whether this functional divergence correlates with CaVβ ability to form dimers when associated to CaVα1 remains controversial. Although it is well established that a single CaVβ molecule or β-GK alone fully reconstitutes modulation of HVA channels (18, 49), a recent report suggests that CaVβ oligomers can also bind to the channel and augment current density (41). The authors attributed this current increase to changes in the functional properties of the channels that they correlate to CaVβ-induced oligomerization induced by a β-GK fragment. Alternative scenarios are that additional monomeric CaVβ increases the fraction of the CaV1.2 subunit complexed with β-subunit or recruits CaVα1-CaVβ channel complexes to the plasma membrane, both augmenting current density (49). Unfortunately, the lack of quantification of the number of channels in the plasma membrane does not allow unambiguous distinction between alterations in channel function or expression.

The number of binding partners and functions of CaVβ is rapidly increasing (4, 50). Very distantly related proteins have been shown to associate with CaVβ, including Rem, Rad, kir/Gem (RGK) GTPases, Ahnak, RIM1, bestrophin, heterochromatin protein 1, a yet to be identified member of intracellular calcium stores, and dynamin. Although the exact sequence of events for the switch between CaVα1-CaVβ and the channel to the CaVβ2-dynamin complex and the physiological signal that triggers dissociation of the β-subunit from its traditional CaVα1 partner have yet to be established, we do know that α1-β interaction is indeed reversible (25). This fact by itself predicts the presence of free β-subunit that can then interact with other ligands besides CaVα1.

In promiscuous proteins, specific associations are often regulated by their structural flexibility. This type of regulation is well documented for the classical members of the membrane-associated guanylate kinase family in which association of ligands with other regions of the membrane regulates GK binding (51–55). The proposed mechanism is that ligands influence SH3-GK intramolecular interactions producing a conformational change within the GK domain that changes the affinity for its partners. For example, upon binding to calmodulin, the synapse-associated protein SAP97 weakens the SH3-GK interaction allowing the accessibility of GK by other proteins (56). Similarly, the SH3-GK interaction that also exists in the β-protein (57–59) may be favored by binding with high affinity to the α1-subunit. Thus, when complexed with the channel, CaVβ intramolecular interactions dominate over intermolecular associations. This is consistent with the FRET analysis showing that dimer formation of CaVβ is reduced in the presence of the α1-subunit. On the contrary, by binding to the SH3 domain,
Dimerization of SH3 Domain Mediates β-subunit Endocytosis

dynamin could weaken intramolecular interactions and favors dimer formation and dissociation from the channel. Our study suggests that dimerization contributes to the functional versatility of CaV β and represents the switch from channel activator to channel internalizer (Fig. 6). Dimerization would endow CaV β with an additional handle to expand the dynamic range over which this protein controls the spatio-temporal profile of calcium signals. After all, the modular architecture of CaV β appears very well suited to orchestrate calcium signaling.

One point that needs to be addressed directly is whether dynamin bind to CaV β when it is complexed to the α-subunit. It would be also interesting to know whether post-translational modifications regulate SH3/GK interactions (60).

The existence of multiple pathways in the regulation of calcium currents by CaV β imposes new theoretical considerations for therapy design. Several evidences suggest that alterations in CaV β underline some forms of myocardial dysfunction (61–64), and so far, gene therapy to inhibit CaV β has been considered only in correlation to an electrophysiological phenotype (65).

Acknowledgments—We thank Dr. Christoph Fahlke for insightful discussions and Rodrigo Neely for reading the manuscript.

REFERENCES

1. Catterall, W. A. (2000) Annu. Rev. Cell Dev. Biol. 16, 521–555
2. Arikath, J., and Campbell, K. P. (2003) Curr. Opin. Neurobiol. 13, 298–307
3. Dolphin, A. C. (2003) J. Bioenerg. Biomembr. 35, 599–620
4. Hidalgo, P., and Neely, A. (2007) Cell Calcium 42, 389–396
5. Berggren, P. O., Yang, S. N., Murakami, M., Ekanayake, A. M., Uhles, S., Köhler, M., Moede, T., Fernström, A., Appelsgaard, J. B., Aspinwall, C. A., Zaitsev, S. V., Larsson, O., de Vargas, L. M., Fecher-Trost, C., Weisgerber, P., Ludwig, A., Leibiger, B., Junti-Berggren, L., Barker, C. I., Gromada, J., Freichel, M., Leibiger, I. B., and Flockerzi, V. (2004) Cell 119, 273–284
6. Hibino, H., Pironkova, R., Onwumere, O., Roussel, M., Charnet, P., Hudspeth, A. J., and Lesage, F. (2003) Proc. Natl. Acad. Sci. U.S.A. 100, 307–312
7. Gonzalez-Gutierrez, G., Miranda-Laferte, E., Neely, A., and Hidalgo, P. (2007) J. Biol. Chem. 282, 2156–2162
8. Chen, Y. H., Li, M. H., Zhang, Y., He, L. L., Yamada, Y., Fitzmaurice, A., Shen, Y., Zhang, H., Tong, L., and Yang, J. (2004) Nature 429, 675–680
9. Opatowsky, Y., Chen, C. C., Campbell, K. P., and Hirsch, J. A. (2004) Neuron 42, 387–399
10. Van Petegem, F., Clark, K. A., Chatelain, F. C., and Minor, D. L., Jr. (2004) Nature 429, 671–675
11. Hinshaw, J. E. (2000) Annu. Rev. Cell Dev. Biol. 16, 483–519
12. Praefcke, G. J., and McMahon, H. T. (2004) Nat. Rev. Mol. Cell Biol. 5, 133–147
13. Gout, I., Dhand, R., Hiles, I. D., Fry, M. J., Panayotou, G., Das, P., Truong, O., Totty, N. F., Hsuam, J., Booker, G. W., and, (1993) Cell 75, 25–36
14. David, C., McPherson, P. S., Mundigl, O., and de Camilli, P. (1996) Proc. Natl. Acad. Sci. U.S.A. 93, 331–335
15. Shupliakov, O., Low, P., Grabs, D., Gad, H., Chen, H., David, C., Takei, K., De Camilli, P., and Brodin, L. (1997) Science 276, 259–263
16. Mayer, B. J. (2001) J. Cell Sci. 114, 1253–1263
17. Pragnell, M., De Waard, M., Mori, Y., Tanabe, T., Snutch, T. P., and Campbell, K. P. (1994) Nature 368, 67–70
18. Gonzalez-Gutierrez, G., Miranda-Laferte, E., Nothmann, D., Schmidt, S., Neely, A., and Hidalgo, P. (2008) Proc. Natl. Acad. Sci. U.S.A. 105, 14198–14203
19. Dzhura, I., and Neely, A. (2003) Biophys. J. 85, 274–289
20. Kaneyevsky, N., and Dascal, N. (2006) J. Gen. Physiol. 128, 15–36
21. Herzig, S., Khan, I. F., Gründer, D., Matthes, J., Ludwig, A., Michels, G., Hoppe, U. C., Chaudhuri, D., Schwartz, A., Yue, D. T., and Hullin, R. (2007) FASEB J. 21, 1527–1538
22. Wigge, P., Köhler, K., Vallis, Y., Doyle, C. A., Owen, D., Hunt, S. P., and McMahon, H. T. (1997) Mol. Biol. Cell 8, 2003–2015
23. Kristensen, O. N., Guenat, S., Dar, I., Allaman-Pillet, N., Abbedrahmani, A., Fardaoussi, M., Roduit, R., Maurer, F., Beckmann, J. S., Kastrup, J. S., Gajhede, M., and Bonny, C. (2006) EMBO J. 25, 785–797
24. Harkiolaki, M., Gilbert, R. J., Jones, E., and Feller, S. M. (2006) Structure 14, 1741–1753
25. Hidalgo, P., Gonzalez-Gutierrez, G., García-Olivares, J., and Neely, A. (2006) J. Biol. Chem. 281, 24104–24110
26. Neely, A., García-Olivares, J., Voswinckel, S., Horstkott, H., and Hidalgo, P. (2004) J. Biol. Chem. 279, 21689–21694
27. Kobe, F., Renner, U., Woehler, A., Włodarczyk, J., Papusheva, E., Bao, G., Zeug, A., Richter, D. W., Neher, E., and Ponomaskin, E. (2008) Biochim. Biophys. Acta 1788, 1503–1516
28. Włodarczyk, J., Woehler, A., Kobe, F., Ponomaskin, E., Zeug, A., and Neher, E. (2008) Biophys J. 94, 986–1000
29. Woehler, A., Włodarczyk, J., and Ponomaskin, E. G. (2009) Glycocolon. J, 26, 749–756
30. Niepmann, M., and Zheng, J. (2006) Electrophoresis 27, 3949–3951
31. Schägger, H., Cramer, W. A., and von Jagow, G. (1994) Anal. Biochem. 217, 220–230
32. Horiuichi, M., Nica, A., Gomez, J., Ascherf, A., Schmalzing, G., and Betz, H. (2001) Proc. Natl. Acad. Sci. U.S.A. 98, 1448–1453
33. Gendreau, S., Voswinckel, S., Torres-Salazar, D., Lang, N., Heidtmann, H., Deto-Dassen, S., Schmalzing, G., Hidalgo, P., and Fahlke, C. (2004) J. Biol. Chem. 279, 39505–39512
34. Deto-Dassen, S., Schänzler, M., Lauks, H., Martin, I., zu Berenstehn, S. M., Nothmann, D., Torres-Salazar, D., Hidalgo, P., Schmalzing, G., and Fahlke, C. (2008) J. Biol. Chem. 283, 4177–4188
35. McGee, A. W., Nunziato, D. A., Maltez, J. M., Prehoda, K. E., Pitt, G. S., and Feller, S. M. (2006) J. Gen. Physiol. 127, 143–155
36. David, C., McPherson, P. S., Mundigl, O., and de Camilli, P. (1996) J. Cell Biol. 134, 25–36
Dimerization of SH3 Domain Mediates β-subunit Endocytosis

43. Béguin, P., Nagashima, K., Gonoï, T., Shibasaki, T., Takahashi, K., Kashima, Y., Ozaki, N., Geering, K., Iwanaga, T., and Seino, S. (2001) Nature 411, 701–706
44. Yang, T., Xu, X., Kernan, T., Wu, V., and Colecraft, H. M. (2010) J. Physiol. 588, 1665–1681
45. Kim, E., Naisbitt, S., Hsueh, Y. P., Rao, A., Rothschild, A., Craig, A. M., and Sheng, M. (1997) J. Cell Biol. 136, 669–678
46. Takeuchi, M., Hata, Y., Hirao, K., Toyoda, A., Irie, M., and Takai, Y. (1997) J. Biol. Chem. 272, 11943–11951
47. Mathew, D., Gramates, L. S., Packard, M., Thomas, U., Bilder, D., Perri-mon, N., Gorczyca, M., and Budnik, V. (2002) Curr. Biol. 12, 531–539
48. Hanada, T., Lin, L., Tibaldi, E. V., Reinherz, E. L., and Chishti, A. H. (2000) J. Biol. Chem. 275, 28774–28784
49. Dalton, S., Takahashi, S. X., Miriyala, J., and Colecraft, H. M. (2005) J. Physiol. 567, 757–769
50. Buraei, Z., and Yang, J. (2010) Physiol. Rev. 90, 1461–1506
51. Brennan, J. E., Topinka, J. R., Cooper, E. C., McGee, A. W., Rosen, J., Milroy, T., Ralston, H. J., and Bredt, D. S. (1998) J. Neurosci. 18, 8805–8813
52. Tavares, G. A., Panepucci, E. H., and Brunger, A. T. (2001) Mol. Cell 8, 1313–1325
53. Qian, Y., and Prehoda, K. E. (2006) J. Biol. Chem. 281, 35757–35763
54. Reese, M. L., Dakoji, S., Bredt, D. S., and Dötsch, V. (2007) Nat. Struct. Mol. Biol. 14, 155–163
55. Newman, R. A., and Prehoda, K. E. (2009) J. Biol. Chem. 284, 12924–12932
56. Paarmann, I., Spangenberg, O., Lavie, A., and Konrad, M. (2002) J. Biol. Chem. 277, 40832–40838
57. Opatowsky, Y., Chomsky-Hecht, O., Kang, M. G., Campbell, K. P., and Hirsch, J. A. (2003) J. Biol. Chem. 278, 52323–52332
58. Takahashi, S. X., Miriyala, J., and Colecraft, H. M. (2004) Proc. Natl. Acad. Sci. U.S.A. 101, 7193–7198
59. Takahashi, S. X., Miriyala, J., Tay, L. H., Yue, D. T., and Colecraft, H. M. (2005) J. Gen. Physiol. 126, 365–377
60. Sabio, G., Arthur, J. S., Kuma, Y., Peggie, M., Carr, J., Murray-Tait, V., Centeno, F., Goedert, M., Morrice, N. A., and Cuenda, A. (2005) EMBO J. 24, 1134–1145
61. Weissgerber, P., Held, B., Bloch, W., Kaestner, L., Chien, K. R., Fleischmann, B. K., Lipp, P., Flockerzi, V., and Freichel, M. (2006) Circ. Res. 99, 749–757
62. Hullin, R., Matthies, J., von Vietinghoff, S., Bodí, I., Rubio, M., D’Souza, K., Friedrich Khan, I., Rottländer, D., Hoppe, U. C., Mohacsi, P., Schmitke- kert, E., Gilsbach, R., Bünnemann, M., Hein, L., Schwartz, A., and Herzig, S. (2007) PLoS One 2, e292
63. Link, S., Meissner, M., Held, B., Beck, A., Weissgerber, P., Freichel, M., and Flockerzi, V. (2009) J. Biol. Chem. 284, 30129–30137
64. Koval, O. M., Guan, X., Wu, Y., Joiner, M. L., Gao, Z., Chen, B., Grumbach, I. M., Luczak, E. D., Colbran, R. J., Song, L. S., Hund, T. J., Mohler, P. J., and Anderson, M. E. (2010) Proc. Natl. Acad. Sci. U.S.A. 107, 4996–5000
65. Cingolani, E., Ramirez Correa, G. A., Kizana, E., Murata, M., Cho, H. C., and Marbán, E. (2007) Circ. Res. 101, 166–175