Caldendrin, a Neuron-specific Modulator of Ca\textsubscript{v}1.2 (L-type) Ca\textsuperscript{2+} Channels*

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EF-hand Ca\textsuperscript{2+}-binding proteins such as calmodulin and CaBP1 have emerged as important regulatory subunits of voltage-gated Ca\textsuperscript{2+} channels. Here, we show that caldendrin, a variant of CaBP1 enriched in the brain, interacts with and distinctly modulates Ca\textsubscript{v}1.2 (L-type) voltage-gated Ca\textsuperscript{2+} channels relative to other Ca\textsuperscript{2+}-binding proteins. Caldendrin binds to the C-terminal IQ-domain of the pore-forming \(\alpha_1\)-subunit of Ca\textsubscript{v}1.2 (\(\alpha_1.2\)) and competitively displaces calmodulin and CaBP1 from this site. Compared with CaBP1, caldendrin causes a more modest suppression of Ca\textsuperscript{2+}-dependent inactivation of Ca\textsubscript{v}1.2 through a different subset of molecular determinants. Caldendrin does not bind to the N-terminal domain of \(\alpha_1.2\), a site that is critical for functional interactions of the channel with CaBP1. Deletion of the N-terminal domain inhibits CaBP1, but spares caldendrin modulation of Ca\textsubscript{v}1.2 inactivation. In contrast, mutations of the IQ-domain abolish physical and functional interactions of caldendrin and Ca\textsubscript{v}1.2, but do not prevent channel modulation by CaBP1. Using antibodies specific for caldendrin and Ca\textsubscript{v}1.2, we show that caldendrin commounprecipitates with Ca\textsubscript{v}1.2 from the brain and colocalizes with Ca\textsubscript{v}1.2 in somatodendritic puncta of cortical neurons in culture. Our findings reveal functional diversity within related Ca\textsuperscript{2+}-binding proteins, which may enhance the specificity of Ca\textsuperscript{2+} signaling by Ca\textsubscript{v}1.2 channels in different cellular contexts.

Caldendrin is a Ca\textsuperscript{2+}-binding protein similar to calmodulin (CaM)\textsuperscript{2} that is localized in neuroendocrine cells (1) and subpopulations of neurons in the brain and retina (2–7). Biochemical analyses indicate caldendrin is tightly associated with the postsynaptic cytomatrix of excitatory synapses (2, 8), where its concentration increases following kainate-induced epileptic seizures in rats (9). These findings suggest a role for caldendrin in regulating postsynaptic signal transduction, perhaps in response to neuronal activity. Like CaM, caldendrin possesses EF-hand Ca\textsuperscript{2+} binding motifs and may regulate the activity of effector molecules in a Ca\textsuperscript{2+}-dependent manner (2, 10). Although CaM interacts with and modulates numerous ion channels and neurotransmitter receptors (11–13), the molecular targets of caldendrin are largely unknown.

Caldendrin is a splice variant of CaBP1 (14), a Ca\textsuperscript{2+}-binding protein that directly regulates voltage-gated Ca\textsuperscript{2+} channels. Like CaM, CaBP1 alters the properties of Ca\textsubscript{v}2.1 (P/Q-type) and Ca\textsubscript{v}1.2 (L-type) voltage-gated Ca\textsuperscript{2+} channels (15–17). CaBP1 and CaM bind to similar sites in the main \(\alpha_1\)-subunit of Ca\textsubscript{v}2.1 and Ca\textsubscript{v}1.2, but the functional consequences of these interactions are different. Whereas CaM contributes to a Ca\textsuperscript{2+}-dependent enhancement (facilitation) of Ca\textsubscript{v}2.1 function (18–20), CaBP1 accelerates inactivation of these channels independent of Ca\textsuperscript{2+} (15). For Ca\textsubscript{v}1.2, CaM mediates Ca\textsuperscript{2+}-dependent inactivation (21–23), while CaBP1 stabilizes channel opening (16, 17).

Although both CaBP1 and caldendrin variants are expressed in the brain (3, 14), several lines of evidence suggest caldendrin may have different regulatory functions than CaBP1 and CaM. First, while the C-terminal-half of caldendrin is identical to that in CaBP1, the N-terminal half of caldendrin is unique and lacks a site for myristoylation (2). Because myristoylation of CaBP1 is required for its ability to enhance inactivation of Ca\textsubscript{v}2.1 (24), the absence of this modification may confer caldendrin with distinct regulatory capabilities. Second, caldendrin mRNA has been detected in different neuronal populations than that for CaBP1 by \textit{in situ} hybridization (3). These findings raise the possibility that caldendrin may differentially modulate voltage-gated Ca\textsuperscript{2+} channels in separate subgroups of neurons in the brain.

Like caldendrin, Ca\textsubscript{v}1.2 Ca\textsuperscript{2+} channels are prominently localized in somatodendritic compartments in neurons (25) where they mediate Ca\textsuperscript{2+} signals important for a variety of processes including synaptic plasticity (26–30). Based on the similar cellular and subcellular distribution of caldendrin and Ca\textsubscript{v}1.2 in the brain, we investigated the significance of caldendrin as a modulator of Ca\textsubscript{v}1.2 Ca\textsuperscript{2+} channels. We show that caldendrin physically and functionally interacts with Ca\textsubscript{v}1.2 in unexpectedly different ways than CaBP1. Caldendrin associates with Ca\textsubscript{v}1.2 in the brain and colocalizes with Ca\textsubscript{v}1.2 in somatodendritic compartments of isolated neurons. Our results suggest a novel role for caldendrin in regulating Ca\textsuperscript{2+} signals and further underscore the importance of such neuron-specific Ca\textsuperscript{2+}-binding proteins in conferring heterogeneous modes of target regulation.
EXPERIMENTAL PROCEDURES

Constructs and Molecular Biology—For electrophysiological and biochemical experiments, Ca_{1.2} subunit cDNAs were α1.2 (rcbl1), β_{1A}, and α_{1.2} (31–33) all cloned in pcDNA3.1+ (Invitrogen). FLAG-α1.2, FLAG-α1.2_Q-EE, FLAG-α1.2 ANT, CaBPs/pcDNA3.1+, CaBPI/pcDNA3.1+, His_{11.2}CaBP1-L/pET30b, and GST-α1.2 constructs were described previously (15–17). A caldendrin cDNA corresponding to the published sequence (GenBank™ Y17408) was amplified by PCR as two fragments corresponding to nucleotides 1–411 and 387–897 from rat brain cDNA (Clontech) with the following primers (non-homologous nucleotides are in lowercase): AL86 (gcaTGAGCTCGACATCGCCAAGAGC) and AL88 (GGCGACTGTTTGAGGAAACGAGTGCCCTGCGGCGGC). AL89 incorporated a silent BamHI site (underlined). The PCR fragments were cloned separately into PCR-4-TOPO (Invitrogen), and the C-terminal fragment was confirmed by DNA sequencing prior to mammalian cell expression. His_{11.2}-caldendrin was constructed by subcloning into EcoRI sites of pcDNA3.1+ (Invitrogen) and confirmed by DNA sequencing prior to mammalian cell expression. His_{11.2}-caldendrin was constructed by subcloning into EcoRI sites of pTrcHisb (Invitrogen).

Preparation and Purification of Caldendrin and α1.2 Antibodies—A GST fusion protein containing amino acids 1–135 of rat caldendrin was generated by subcloning the corresponding cDNA into BamHI and XhoI sites of pGEX-4T-1. The fusion protein was expressed in bacteria, purified according to standard protocols, and used for immunization of rabbits by a commercial source (Covance Research Products Inc., Denver, PA). For α1.2 antibodies, rabbit antiserum was generated (Prosci, Inc., Poway, CA) against a peptide corresponding to a sequence in rat α1.2 (C KYT TKI NMD DLQ PSE NED KS) used previously for the generation of α1.2-specific antibodies (25). The α1.2 antibodies were specific in that they recognized α1.2 in transfected cells and in brain lysate by Western blot, immunoprecipitation, and immunocytochemistry (not shown). Caldendrin and α1.2 antibodies were affinity-purified on columns containing the corresponding immunogen prior to use.

Binding Assays—HEK293T cells were maintained in Dulbecco’s modified Eagle’s medium with 10% fetal bovine serum at 37 °C in a humidified atmosphere under 7% CO_{2}. Cells were grown to ~70–80% confluence and transfected with Gene Porter reagent (Gene Therapy Systems, San Diego, CA). Cells plated on 150-mm dishes were transfected with 10 μg of caldendrin or CaBP1-S cDNA. Two days later, cells were homogenized in 1 ml of ice-cold lysis buffer (20 mM HEPES, 100 mM NaCl, 1.0% Triton X-100, with protease inhibitors: 17 μg/ml phenylmethylsulfonyl fluoride, 1 μg/ml benzamidine, 1 μg/ml aprotinin, 1 μg/ml leupeptin, and 1 μg/ml pepstatin A, pH 7.4), and membrane proteins were solubilized by rotating at 4 °C for 30 min. Insoluble material was removed by ultracentrifugation at 100,000 × g for 30 min, and the supernatant was used immediately or aliquoted and stored at −80 °C. GST-α1.2 fusion proteins were immobilized on glutathione-agarose beads and incubated with purified CaM (5 μg, Sigma-Aldrich) or transfected cell lysates in a total volume of 1 ml with binding buffer (TBS-buffered saline (TBS, 20 mM Tris, 150 mM NaCl, pH 7.3), 0.1% Triton X-100, and protease inhibitors) containing either 2 mM CaCl_{2} or 10 mM EGTA at 4 °C, rotating for 4 h. The beads were washed three times with binding buffer (1 ml), and bound proteins were detected by SDS-PAGE and transferred to nitrocellulose. Caldendrin or CaM was detected by Western blot with rabbit polyclonal antibodies against CaBP1/caldendrin (UW72, 1:1000 (14)) or mouse monoclonal anti-CaM antibodies (1:500, Millipore, Temecula, CA), respectively. Blots were processed with horseradish peroxidase-conjugated secondary antibodies (anti-rabbit IgG 1:4000, anti-mouse IgG 1:4000) and reagents for enhanced chemiluminescent detection (Amer sham Biosciences).

For competitive binding assays, His_{11.2}-caldendrin and His_{11.2}-CaBP1-L were expressed in BL21 Escherichia coli, purified, and protein concentrations were determined by BCA assay (Pierce). GST-α1.2 fragments immobilized on glutathione beads were preincubated for 10 min at 4 °C with 0.05 μM His_{11.2}-caldendrin, His_{11.2}-CaBP1-L, or purified CaM (Sigma-Aldrich) in 0.5 ml of binding buffer (TBS, 0.1% Triton X-100, 2 mM CaCl_{2}, and protease inhibitors). Varying concentrations of the competing protein were added and the reaction continued for 2 h at 4 °C. Samples were washed three times in binding buffer, subjected to SDS-PAGE and Western blotting with rabbit polyclonal antibodies to His_{11.2} (1:1000, Santa Cruz Biotechnology), pan-CaBP1, or CaM antibodies as described above.

Coimmunoprecipitation Assays—For coimmunoprecipitation from transfected cells, HEK293T cells were transfected with equimolar amounts of FLAG-α1.2 (wild-type or with ΔNT or IQ-EE mutations), α1.2, β_{1A}, and caldendrin with or without CaBP1-L. After 42 h, cells were lysed, and membrane proteins solubilized in lysis buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1% Nonidet P-40, 0.25% sodium deoxycholate, 1 mM EDTA, and protease inhibitors) for 30 min at 4 °C, subjected to centrifugation at 1,000 × g for 5 min. The resulting supernatant was incubated with caldendrin or α1.2 antibodies (8 μg) for 1 h, followed by the addition of preswollen protein A-Sepharose (50 μl, 50% slurry) for 2 h and washed three times in lysis buffer. Immunoprecipitated proteins were detected by Western blotting with antibodies against α1.12 (1:5000) and caldendrin (1:4000) or CaBP1 (UW72, 1:4000).

For immunoprecipitation from brain, frozen brains from adult male Sprague-Dawley rats were purchased from Pel-Freez (Rogers, AK). For one experiment, one brain was homogenized in ice-cold buffer (4 mM HEPES, 1 mM EDTA, and 0.32 mM sucrose, pH 7.4, with protease inhibitors) and large debris and nuclei were removed by centrifugation at 1000 × g for 5 min. The supernatant was collected and subjected to centrifugation at 100,000 × g for 30 min. The resulting membrane pellet was resuspended in ice-cold solubilization buffer (8 ml; 1% Triton X-100, 20 mM EDTA, 10 mM EGTA, 10 mM Tris, pH 7.4, and protease inhibitors) for 30 min on ice and insoluble material removed by centrifugation at 100,000 × g for 30 min. The supernatant (0.5 ml) was incubated with antibodies against caldendrin or control rabbit IgG (Jackson Immuno Research, West Grove, PA) (10 μg) for 4 h, rotating at 4 °C. Pre-swollen proteins were incubated with His_{11.2}-caldendrin or His_{11.2}-CaBP1-L at 4 °C for 30 min. The beads were washed three times with binding buffer (1 ml), and bound proteins were detected by SDS-PAGE and transferred to nitrocellulose. Caldendrin or CaM was detected by Western blot with rabbit polyclonal antibodies against CaBP1/caldendrin (UW72, 1:1000 (14)) or mouse monoclonal anti-CaM antibodies (1:500, Millipore, Temecula, CA), respectively. Blots were processed with horseradish peroxidase-conjugated secondary antibodies (anti-rabbit IgG 1:4000, anti-mouse IgG 1:4000) and reagents for enhanced chemiluminescent detection (Amer sham Biosciences).

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Channels

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protein A-Sepharose (50 μl of 50% slurry) was washed three times with solubilization buffer and added to immunoprecipitation reactions. After further incubation, rotating at 4 °C overnight, the beads were washed, and immunoprecipitated proteins were detected by Western blotting. For Fig. 7, C and D, the blot was incubated in stripping buffer (62.5 mM Tris, 1% SDS, 3.1% dithiothreitol) for 30 min at 50 °C prior to re-probing with CaM or CaBP1 antibodies.

**Immunocytochemistry of Primary Neurons in Culture**—Primary cultures of neurons were prepared from neocortical tissue dissected from rat embryos (E19). The tissue was incubated with papain for 1 h at 37 °C and triturated in inactivation solution (minimum essential medium, MEM (Invitrogen) and 10% fetal bovine serum). The resulting cell suspension was plated at a density of ~300,000 neurons per 60-mm plate containing glass coverslips that were precoated with poly-D-lysine. Neurons were maintained in medium containing MEM, 1% pyruvate, 0.6% dextrose, 5% fetal bovine serum, 1X B-27 (Invitrogen), 0.5 mM penicillin/streptomycin/glutamine, and 0.001% MITO and serum extender (BD Biosciences, San Jose, CA). After 16–21 days in culture, cells were fixed with 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.3) for 20 min, rinsed in TBS, and blocked for 30 min in TBS containing 10% NGS and 0.1% Triton X-100. All antibodies were diluted in TBS containing 2.5% NGS and 0.1% Triton X-100, and cells were rinsed three times for 5 min following each incubation period. All fluorescent secondary antibodies were obtained from Jackson ImmunoResearch. After blocking, cells were incubated with caldendrin antibodies (1:100) for 1 h at room temperature and after rinsing, with rhodamine-conjugated anti-rabbit Fab fragments (1:200) for 30 min. Before double-labeling, cells were blocked with goat anti-rabbit Fab fragments (1:200) for 20 min. Cells were then incubated with α1,2 antibodies (1:100) overnight at 4 °C and after rinsing, with biotinylated anti-rabbit IgG (1:1300) for 1 h and subsequently with FITC-avidin (1:1000, Vector Laboratories, Burlingame, CA). Coverslips were washed and mounted on glass slides in Vectashield (Vector Laboratories) prior to viewing with a Zeiss (Oberkochen, Germany) LSM510 Meta confocal microscope. Image processing was performed with Zeiss LSM Image Browser and Adobe Photoshop (Adobe Systems Inc., San Jose, CA) software and quantitatively analyzed using Metamorph software (Universal Imaging Co., Downingtown, PA). All images were thresholded to similar levels to obtain representative images. Fluorescent signal colocalization was determined from optical sections (1 μm) taken from the central plane of the neuron. Pixels containing both fluorescent signals were considered colocalized, and this value was expressed as a percentage of the total number of pixels positive for one fluorochrome.

**Electrophysiological Recordings**—HEK293T cells were transfected with a total of 5 μg of DNA (Ca,1.2 subunits: α,1,2, β,3, δ,6) including 0.2 μg of a pEGFP-N1, caldendrin/pEGFPN1, or CaBP1-S/pEGFPN1 for fluorescent detection of transfected cells. 24–48 h after transfection, whole cell patch clamp recordings of transfected cells were acquired with a HEKA Elektronik (Lambrecht/Pfalz, Germany) EPC-9 patch clamp amplifier. Data acquisition and leak subtraction using a P/4 protocol were performed with Pulse software (HEKA Elektronik). Extracellular recording solutions contained (in mM): 150 Tris, 2 MgCl<sub>2</sub>, and 10 or 20 CaCl<sub>2</sub> or BaCl<sub>2</sub>. Intracellular solutions contained (in mM): 140 N-methyl-d-glucamine, 10 HEPES, 2 MgCl<sub>2</sub>, 2-MgATP, and 5 EGTA. The pH of the recording solutions was adjusted to 7.3 with methanesulfonic acid. Series resistance was ~2–4 MΩ, compensated up to 70%. Signals were filtered at 2 kHz and sampled at 10–20 kHz. I-V curves were fit by Equation 1,

\[
I = g(V - E)/(1 + \exp[(V_{1/2} - V)/k])
\]

where \(g\) is maximum conductance, \(V\) is test voltage, \(E\) is apparent reversal potential, \(V_{1/2}\) is half-maximal activation, and \(k\) is the slope factor. Data were analyzed using Igor software (WaveMetrics, Lake Oswego, OR), and graphs plotted with SigmaPlot (SPSS, Chicago, IL). Statistical comparisons between groups were made by Student’s t test.

**RESULTS**

Caldendrin Binds to the α,1.2-Subunit of Ca,1.2—CaM binding to a well-characterized IQ-domain and pre-IQ-domain in the cytoplasmic C-terminal region of the Ca,1.2 α,1.2 subunit (α,1.2) mediates a negative feedback regulation of incoming Ca<sup>2+</sup> ions known as Ca<sup>2+</sup>-dependent inactivation (CDI) (21, 23, 34–39).

Although CaBP1 also binds to the IQ-domain, we have shown that CaBP1 binding to the N-terminal domain (NT) of α,1.2 is essential for suppressing CDI (17). To gain insight into how caldendrin might regulate Ca,1.2, we identified the sites in α,1.2 with which caldendrin interacts in pull-down assays with GST-tagged fragments of the cytoplasmic domains of α,1.2 (Fig. 1A). As we have shown previously, CaM and CaBP1 interacted with the NT, but only CaBP1 bound the NT both with and without Ca<sup>2+</sup> (Fig. 1B). Despite its similarity to CaBP1, caldendrin did not bind to the NT either in the presence or absence of Ca<sup>2+</sup>. This result demonstrates the specificity with which even highly related Ca<sup>2+</sup>–binding proteins can interact with the same target sequence. Like CaBP1 (16), caldendrin bound both with and without Ca<sup>2+</sup> to a C-terminal fragment of α,1.2 that included both the IQ and Pre-IQ region (CT1, Fig. 1, A and C). The absence of caldendrin and CaBP1 binding to a fragment downstream of this region (CT2, Fig. 1A) confirmed the specificity of the interaction. Further analyses showed that like CaBP1, caldendrin binds to the Pre-IQ sites (CT7) both with and without Ca<sup>2+</sup> and to the IQ-domain (CT6) stronger in the presence of Ca<sup>2+</sup> (Fig. 1C). These results suggested that caldendrin and CaBP1 constitutively associate with the Pre-IQ region and exhibit some Ca<sup>2+</sup>-dependent binding to the IQ-domain. Under the same assay conditions where Ca<sup>2+</sup>-independent binding was assessed in the presence of EGTA, CaM showed strictly Ca<sup>2+</sup>-dependent binding to CT1 and CT6 and weak Ca<sup>2+</sup>-independent binding to CT7 (Fig. 1C). Previous studies showed that in biochemical assays, CaM does not bind to Pre-IQ or IQ sites in the complete absence of Ca<sup>2+</sup>, but requires nanomolar concentrations of Ca<sup>2+</sup> (35). Thus, our results highlight differences in the Ca<sup>2+</sup> requirements for CaBP1/caldendrin and CaM association with the α,1.2 C-terminal domain.

To determine if caldendrin associated with similar determinants within the Pre-IQ and IQ-domains as CaBP1 and CaM,
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A

B

C

FIGURE 1. Caldendrin binds to a subset of sites in α\textsubscript{1.2} that interact with CaM and CaBP1. A, schematic of rat brain α\textsubscript{1.2} showing EF-hand (EF), Pre-IQ, and IQ-domains implicated in CDI of Ca\textsubscript{v}1.2. N- and C-terminal regions tested for binding caldendrin, CaBP1, or CaM are shown (NT, CT1, CT2, CT6, CT7). Parentheses indicate amino acid boundaries. B, GST-tagged α\textsubscript{1.2} N-terminal fragment (NT) in A was coupled to glutathione-agarose beads and incubated with CaBP1 or caldendrin (CD) from transfected cell lysates or purified CaM. C, binding of GST-tagged α\textsubscript{1.2} C-terminal fragments to caldendrin (CD) or CaBP1 from transfected cell lysates or purified CaM. For B and C, assays contained 2 mM Ca\textsuperscript{2+} (+) or 10 mM EGTA (−). Bound proteins were detected by Western blotting with pan-CaBP1 (for CD and CaBP1) or CaM antibodies. The Ponceau stain of the blots indicate levels of GST fusion protein used in the assay. Results shown are representative of at least three experiments.

Caldendrin Inhibits Ca\textsuperscript{2+}-dependent Inactivation of Ca\textsubscript{v}1.2 Channels—CaBP1 binding to the NT of α\textsubscript{1.2} blocks CDI of Ca\textsubscript{v}1.2 by markedly slowing inactivation of Ca\textsubscript{v}1.2 Ca\textsuperscript{2+} currents (17). Because caldendrin interacts with the C-terminal IQ- and Pre-IQ-domains, but not the NT (Fig. 1, B and C), caldendrin may have effects on Ca\textsubscript{v}1.2 inactivation that are distinct from those of CaBP1. We tested this by comparing CDI in whole cell patch-clamp recordings of HEK293T cells transfected with Ca\textsubscript{v}1.2 alone and cotransfected with caldendrin. Inactivation of currents evoked by sustained depolarizations was measured as I\textsubscript{res}/I\textsubscript{pk}, which was the amplitude of the current at the end of the test pulse normalized to that for the peak current. With Ca\textsuperscript{2+} as the charge carrier, inactivation of Ca\textsubscript{v}1.2 currents was rapid (small I\textsubscript{res}/I\textsubscript{pk}, Fig. 3, A and B) and results from interactions of the channels with CaM that is endogenously expressed in HEK293T cells (22). Inactivation of Ca\textsubscript{v}1.2 is slow when Ba\textsuperscript{2+} is used as the permeant ion (large I\textsubscript{res}/I\textsubscript{pk}, Fig. 3, A and B) because Ba\textsuperscript{2+} substitutes poorly for Ca\textsuperscript{2+} in binding to CaM (42). The difference between I\textsubscript{res}/I\textsubscript{pk} for Ca\textsuperscript{2+} currents (I\textsubscript{Ca}) and Ba\textsuperscript{2+} currents (I\textsubscript{Ba}) therefore provides a measure of CDI, which was large in cells transfected with Ca\textsubscript{v}1.2 alone (Fig. 3B). In comparison, CDI was significantly decreased (~31%) in cells cotransfected with caldendrin (p < 0.01; Fig. 3B). The inhibitory effect of caldendrin on CDI was not related to changes in voltage-dependent activation because there were no significant differences in the parameters (V\textsubscript{1/2}, k) for I\textsubscript{Ca} or I\textsubscript{Ba} current-voltage relationships for Ca\textsubscript{v}1.2 alone or Ca\textsubscript{v}1.2+caldendrin (p > 0.5; Fig. 3, C and D). The effect of caldendrin differed from that of CaBP1 in that CDI was moderately inhibited and not completely abolished as with CaBP1 (Fig. 3, A and B). I\textsubscript{Ca} still inactivated significantly more than I\textsubscript{Ba} (~45%) in cells cotransfected with caldendrin (p < 0.01, Fig. 3, A and B). In contrast, there was no significant difference in I\textsubscript{Ca} and I\textsubscript{Ba} inactivation in cells cotransfected with CaBP1 (p = 0.34, Fig. 3, A and B).

To evaluate the difference between caldendrin and CaBP1 on CDI during more physiological stimuli, inactivation of I\textsubscript{Ca} and I\textsubscript{Ba} was measured during trains of repetitive depolarizations (Fig. 4). Inactivation was manifest as a decrease in the fractional current, which was the amplitude of each test current normalized to that for the first in the train. CDI was measured as the decrease in fractional I\textsubscript{Ca} and I\textsubscript{Ba} for the last 10 pulses of the train. Compared with cells transfected with Ca\textsubscript{v}1.2 alone, cells cotransfected with Ca\textsubscript{v}1.2+caldendrin showed significantly less inactivation of I\textsubscript{Ca} (~33%, p < 0.01) and increased inactivation of I\textsubscript{Ba} (~3%, p < 0.01) during the repetitive stimulation protocol. As a consequence, CDI was significantly less (~52%, p < 0.01) in cells cotransfected with caldendrin than in cells with Ca\textsubscript{v}1.2 alone (Fig. 4, A and B). As was the case during the sustained depolarization protocol (Fig. 3, A and B), moderate CDI was still evident in cells cotransfected caldendrin, as I\textsubscript{Ca} inactivated more significantly than I\textsubscript{Ba} (22%, p < 0.01, Fig. 4B). In contrast, and as we have shown previously (16), in cells cotransfected with CaBP1, CDI was abolished in that the amplitudes of I\textsubscript{Ca} and I\textsubscript{Ba} did not significantly differ at the end of the pulse train (p = 0.40). Our results demonstrate functional heterogeneity in the effects of CaBP1 and caldendrin on Ca\textsubscript{v}1.2 inactivation, perhaps as a consequence of the distinct sites on the channel with which each interacts.

We therefore compared the impact of alterations in the α\textsubscript{1.2} sites with which caldendrin and/or CaBP1 associate (Fig. 1). Since both CaBP1 and caldendrin bind to the IQ-domain (Figs. 1C and 2D), we first analyzed the role of this region for the modulatory effects of these CaBPs on CDI. The IQ residues make functionally
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**FIGURE 2.** Caldendrin competes with CaM and CaBP1 for binding to the \alpha\textsubscript{1.2} C-terminal domain. Immobilized GST-CT1 (A and B) or GST-CT6 (C and D) (~1 \(\mu\)g) as described in the legend to Fig. 1, was incubated with a fixed concentration (0.05 \(\mu\)M) of purified His-tagged caldendrin (CD) or His-tagged CaBP1 (B and D, left panels), or CaM (A and C, left panels) and varying concentrations of the competing proteins as indicated. Bound proteins were detected on the same blot by Western blotting with anti-His antibodies (for CD) or CaM antibodies (A and C). The slower mobility of CD compared with CaBP1 allowed simultaneous detection of both proteins on same blot with pan-CaBP1 antibodies (B and D). Assays contained 2 mM Ca\textsuperscript{2+}. Results shown are representative of three experiments.

**FIGURE 3.** Caldendrin suppresses CDI of Ca\textsubscript{\textalpha},1.2. A normalized traces represent currents evoked by 1-s pulses from -80 mV to +20 mV (I\textsubscript{Ca}, black) or +10 mV (I\textsubscript{Ba}, gray) in HEK293T cells transfected with Ca\textsubscript{\textalpha},1.2 alone or cotransfected with caldendrin (CD) or CaBP1. B, \textfrac{I\textsubscript{Ca}}{I\textsubscript{Ba}} (right) was calculated as the residual current amplitude at the end of the pulse normalized to the peak current amplitude for I\textsubscript{Ca} (n = 11, Ca\textsubscript{\textalpha},1.2; n = 10, +CD; n = 12, +CaBP1) and I\textsubscript{Ba} (n = 12, Ca\textsubscript{\textalpha},1.2; n = 10, +CD; n = 8, +CaBP1). Caldendrin \textsuperscript{-}dependent inactivation (CDI) (I\textsubscript{Ca}/I\textsubscript{Ba}, left) is the difference in \textfrac{I\textsubscript{Ca}}{I\textsubscript{Ba}} for Ca\textsubscript{\textalpha},1.2 and I\textsubscript{Ba} for cells transfected with Ca\textsubscript{\textalpha},1.2 alone (n = 12) or cotransfected with caldendrin (n = 11) or CaBP1 (n = 12). For CDI\textsubscript{**,**}, \(p < 0.01\) compared with Ca\textsubscript{\textalpha},1.2 alone. For I\textsubscript{Ca}/I\textsubscript{Ba}**, \(p < 0.01\) compared with I\textsubscript{Ca} and D, current-voltage curves for cells transfected with Ca\textsubscript{\textalpha},1.2 alone or cotransfected with caldendrin. I\textsubscript{Ca} or I\textsubscript{Ba} was evoked by 50 ms pulses to varying test voltages from a holding voltage of -80 mV. Current amplitudes were normalized to the largest in the series (I\textsubscript{max}) and plotted against the test voltage. Points represent mean ± S.E. Curve fitting was with the Boltzmann equation and shown for cells transfected with Ca\textsubscript{\textalpha},1.2 alone (smooth line) or cotransfected with CD (dashed line). Values for \textfrac{V}{\textfrac{1}{2}} (mV), \(K_{\text{m}}\), were: 16.93 ± 1.98, -8.43 ± 0.28, n = 8 for Ca\textsubscript{\textalpha},1.2 alone; 22.24 ± 2.44, -8.86 ± 0.29, n = 8 for Ca\textsubscript{\textalpha},1.2 + CD; 1.79 ± 4.09, -7.70 ± 0.23, n = 7 for I\textsubscript{Ca} Ca\textsubscript{\textalpha},1.2 alone; 7.09 ± 4.85, -7.05 ± 0.94, n = 7 for I\textsubscript{Ba} Ca\textsubscript{\textalpha},1.2 + CD. Extracellular solution contained 20 mM Ca\textsuperscript{2+} or Ba\textsuperscript{2+}.

This is evident in the elimination of CDI in channels containing IQ-EE substitutions (\(p < 0.01\) compared with wild-type Ca\textsubscript{\textalpha},1.2, Fig. 5A). These results are in contrast to the residual modulation of Ca\textsubscript{\textalpha},1.2\textsubscript{IQ-EE} by CaBP1. CaBP1 still decreased inactivation of I\textsubscript{Ca} (~23%, \(p < 0.01\)) but also decreased I\textsubscript{Ba} inactivation (~28%) such that net CDI was not different from Ca\textsubscript{\textalpha},1.2\textsubscript{IQ-EE} alone (\(p = 0.47\)). In contrast, cotransfection of caldendrin with Ca\textsubscript{\textalpha},1.2\textsubscript{IQ-EE} did not affect inactivation of I\textsubscript{Ca} (\(p = 0.44\)), I\textsubscript{Ba} (\(p = 0.4\)) or CDI (\(p = 0.92\) compared with Ca\textsubscript{\textalpha},1.2\textsubscript{IQ-EE} alone). While loss of Ca\textsubscript{\textalpha},1.2\textsubscript{IQ-EE} regulation by caldendrin was supported by the failure of caldendrin to coimmunoprecipitate with Ca\textsubscript{\textalpha},1.2\textsubscript{IQ-EE} (Fig. 5C), we have shown previously that the IQ-EE substitution also prevents coimmunoprecipitation of CaBP1 (17). We propose that the IQ-domain is an essential determinant for physical association of CaBP1 and caldendrin with Ca\textsubscript{\textalpha},1.2. However, our electrophysiological data indicate a more significant role for the IQ-domain in mediating the effects of caldendrin compared with CaBP1 on Ca\textsubscript{\textalpha},1.2 inactivation.

To further compare the determinants underlying CaBP1 and caldendrin modulation, we turned to Ca\textsubscript{\textalpha},1.2\textsubscript{NT} channels lacking the first 60 amino acids in \alpha\textsubscript{1.2} as these channels are insensitive to modulation by CaBP1 (17). Because CaBP1 but not caldendrin binds to the \alpha\textsubscript{1.2} N-terminal domain (Fig. 1, A and B), we hypothesized that Ca\textsubscript{\textalpha},1.2\textsubscript{NT} may still be subject to regulation by caldendrin but not CaBP1. Consistent with our previous results (17), CaBP1 did not significantly affect CDI of Ca\textsubscript{\textalpha},1.2\textsubscript{NT} (\(p = 0.14\), Fig. 5B). In contrast, CDI was still significantly decreased in cells cotransfected with caldendrin compared with cells with Ca\textsubscript{\textalpha},1.2\textsubscript{NT} alone (~50%, \(p < 0.01\), Fig. 5B). The ability of caldendrin to directly modulate Ca\textsubscript{\textalpha},1.2\textsubscript{NT} was supported by their coimmunoprecipitation.

relevant contacts with CaM (41, 43) such that their substitution, particularly with hydrophilic glutamate residues (EE), completely abolishes interactions with, and modulation by, CaM (34, 35, 37). from transfected cells (Fig. 5C). These results demonstrate a requirement of the \alpha\textsubscript{1.2} N-terminal domain for regulation by CaBP1 but not caldendrin.
Cav1.2 channels in a physiological context, we developed and determined the extent to which caldendrin might interact with cotransfected with caldendrin (CD) alone (A) or cotransfected with caldendrin (CD) or CaBP1 (C). Representative current traces from the first (left) and last (right) 7 pulses are shown. Dashed line indicates initial current amplitude. Test current amplitudes were normalized to the first in the train (Fractional current) and plotted against time for ICa (filled circles, n = 7 in A, n = 6 in B, n = 5 in C) or IBa (open circles, n = 7 in A, n = 6 in B, n = 6 in C). Extracellular solution contained 10 mM Ca2+ or Ba2+.* p < 0.01.

**FIGURE 4.** Caldendrin inhibits CDI less than CaBP1 during repetitive stimuli. Currents were evoked by 5-ms pulses from −80 mV to +10 mV for ICa or to 0 mV for IBa, at a frequency of 100 Hz in cells transfected with Cav1.2 alone (A) or cotransfected with caldendrin (CD) (B) or CaBP1 (C). Representative current traces from the first (left) and last (right) 7 pulses are shown. Dashed line indicates initial current amplitude. Test current amplitudes were normalized to the first in the train (Fractional current) and plotted against time for ICa (filled circles, n = 7 in A, n = 6 in B, n = 5 in C) or IBa (open circles, n = 7 in A, n = 6 in B, n = 6 in C). Extracellular solution contained 10 mM Ca2+ or Ba2+.* p < 0.01.

**FIGURE 5.** Distinct role of α1.2 NT and IQ-domain for modulation of CDI by caldendrin and CaBP1. A and B, ICa and IBa, and CD were measured as in Fig. 3B for cells transfected with Cav1.2IQ-EE (A) or Cav1.2NT alone or cotransfected with caldendrin (CD) or CaBP1. Representative current traces for ICa (black) and IBa (gray) were shown above. In A, p < 0.01 for comparisons indicated by brackets. In B, * p < 0.01 for CDI compared with Cav1.2NT alone and for ICa/IBa of ICa compared with IBa. C, coimmunoprecipitation of CD with Cav1.2NT but not Cav1.2IQ-EE. HEK293T cells transfected with CD alone (lane 5) or cotransfected with Cav1.2NT (lanes 1 and 2) or Cav1.2IQ-EE (lanes 3 and 4) (FLAG-α, 1.2NT or FLAG-α, 1.2IQ-EE, β2A, α6) were subject to lysis and immunoprecipitation with α1.2 antibodies. Coimmunoprecipitated proteins were detected by Western blotting with FLAG or CD antibodies. Assays were done with 2 mM Ca2+ (+) or 10 mM EGTA (−). Results shown are representative of three experiments.

**Characterization of Caldendrin-specific Antibodies**—To determine the extent to which caldendrin might interact with Cav1.2 channels in a physiological context, we developed and characterized antibodies that would specifically recognize caldendrin and not the other CaBP1 variants in native tissue. These antibodies were targeted against the first 135 amino acids of caldendrin, a sequence that distinguishes caldendrin from the other CaBP1 variants (CaBP1-S, CaBP1-L, Fig. 6A). The specificity of these antibodies was confirmed according to the following criteria. First, the caldendrin antibodies detected a single band of the appropriate size in Western blots of lysates from HEK293T cells transfected with the caldendrin cDNA and not from cells transfected with CaBP1-S or CaBP1-L (Fig. 6B). Western blotting with pan-CaBP1 antibodies confirmed that equal levels of each variant were expressed in the lysates (Fig. 6B). Second, Western blotting of brain lysates revealed a single band consistent in size with caldendrin in transfected cell lysates (Fig. 6C), supporting the specificity of the antibodies for native caldendrin. Third, caldendrin antibodies but not control IgG immunoprecipitated a protein consistent in size with caldendrin but not CaBP1-S or CaBP1-L from rat brain lysates (Fig. 6C). The identity of this immunoprecipitated protein as caldendrin was confirmed by Western blotting with caldendrin and pan-CaBP1 antibodies (Fig. 6C). Finally, in immunocytochemical experiments, caldendrin antibodies labeled HEK293T cells transfected with caldendrin but not CaBP1S or CaBP1L (not shown). These results validated the use of the caldendrin antibodies for specifically labeling caldendrin and not other CaBP1 variants in native preparations.

**Caldendrin Associates and Colocalizes with Brain Cav1.2 Channels**—To evaluate the physiological significance of caldendrin interactions with Cav1.2, we first determined if caldendrin interacted with Cav1.2 channels in the brain. When caldendrin antibodies were used for the immunoprecipitation, α1.2 was found to coimmunoprecipitate with caldendrin (Fig. 7A and B, lane 2). The coimmunoprecipitation was specific in that no α1.2 or caldendrin was detected when a control rabbit IgG was used (Fig. 7A and B, lane 1). The coimmunoprecipitated pro-
teins were consistent in size with recombinant α1.2 and caldendrin in lysates from cotransfected HEK293T cells (Fig. 7A and B, lanes 3 and 4). Western blotting with CaM antibodies revealed that CaM, although present in the brain lysate, was not associated with Cav1.2 channels coimmunoprecipitated by caldendrin antibodies (Fig. 7C, lane 2). Right, Western blot of rat brain lysate (lane 1) with CD antibodies detects single band consistent in size with CD in transfected HEK293T cells (lane 2). Results are representative of two experiments. C, specificity of caldendrin antibodies for brain caldendrin. Brain lysates were subject to immunoprecipitation with caldendrin antibodies (lane 1) or control IgG (lane 2). Immunoprecipitated protein was detected by Western blot with pan-CaBP1 antibodies (left) or caldendrin antibodies (right). Results are representative of three experiments.

Because our competitive binding assays indicated a potential for CaBP1 and caldendrin to interact with the channel simultaneously (Fig. 2B), we also tested if CaBP1 associated with...
brain Ca$_{1.2}$/caldendrin complexes by Western blotting with pan-CaBP1 antibodies (Fig. 7D), which recognize both CaBP1-L and CaBP1-S (Fig. 6B). While neither CaBP1-L or CaBP1-S were coimmunoprecipitated with caldendrin/Ca$_{1.2}$, the CaBP1 variants were also undetectable in the solubilized rat brain lysate (Fig. 7B, lane 3). We previously found that CaBP1-S was enriched in postsynaptic density fractions of rat brain, and coimmunoprecipitated with Ca$_{1.2}$ channels from these preparations (16). Whereas we were unable to coimmunoprecipitate Ca$_{1.2}$ and caldendrin from these postsynaptic density fractions (not shown), the complex was isolated in the present study under milder detergent solubilization conditions (Fig. 7, A and B). Previous studies have shown that CaBP1-L and CaBP1-S are present at lower levels in the brain and not represented in the same subcellular fractions as caldendrin (2, 3).

Therefore, caldendrin and CaBP1 may associate with separate populations of Ca$_{1.2}$ channels in different subcellular domains. To determine the potential for CaBP1 and caldendrin to co-associate with Ca$_{1.2}$, we expressed caldendrin, CaBP1-L, and Ca$_{1.2}$ together in transfected HEK293T cells. In these experiments, CaBP1-L could be detected, although weakly, in Ca$_{1.2}$/caldendrin coimmunoprecipitates (Fig. 7E). These results are consistent with our findings that CaBP1-L and caldendrin can interact simultaneously with the Ca$_{1.2}$ C-terminal domain (Fig. 2). However, whether neuronal Ca$_{1.2}$ channels form complexes with more than one CaBP remains to be determined.

To further confirm the importance of caldendrin/Ca$_{1.2}$ interactions in neurons, we analyzed the extent to which these proteins colocalize in double-label immunofluorescence analyses of cortical neurons in culture. Previous analyses indicate that caldendrin and Ca$_{1.2}$ are both localized in somatodendritic domains of cortical and hippocampal pyramidal cells (3, 8, 25, 44). Consistent with these reports, caldendrin and Ca$_{1.2}$ channel immunoreactivities (CD-IR and Ca$_{1.2}$ IR, respectively) in our experiments were intense and punctate in the soma and dendritic processes of virtually all neurons that were examined (Fig. 8, A–F). Quantitative analyses indicated that CD-IR and Ca$_{1.2}$-IR colocalized more extensively in cell soma than in dendritic processes (Fig. 8, A–F). Of the somatic puncta that were immunolabeled for Ca$_{1.2}$, 61 ± 7% were also labeled with caldendrin antibodies (n = 13 neurons) while 36 ± 6% of Ca$_{1.2}$-immunoreactive puncta in the dendrites (n = 17) also showed CD-IR. In comparison, a larger proportion of total CD-IR overlapped with Ca$_{1.2}$-IR both in the cell soma (82 ± 4%, n = 13) and in the dendrites (58 ± 6%, n = 17). These results suggest that caldendrin may be a primary partner of Ca$_{1.2}$ channels, particularly in the cell soma. The fact that more than half of the Ca$_{1.2}$-immunolabeled puncta in the dendrites were without CD-IR suggests that many Ca$_{1.2}$ channels in these subcellular domains do not interact with caldendrin possibly because of associations with other regulatory molecules, such as CaBP1 or CaM. Finally, the overall stronger colocalization of caldendrin with Ca$_{1.2}$ in the soma than in dendrites suggests that caldendrin may be most important in regulating somatic Ca$_{1.2}$ Ca$^{2+}$ signals and their role in neuronal excitability and gene transcription.

**DISCUSSION**

Our findings provide new insights into the physiological role of caldendrin in neurons. Like CaBP1 and CaM, caldendrin binds to the C-terminal domain of the α$_{1}$ subunit of Ca$_{1.2}$ but has effects on Ca$_{1.2}$ inactivation that differ from those of CaBP1 or CaM. Caldendrin associates and colocalizes with Ca$_{1.2}$ in neurons and therefore may represent a physiologically relevant component of Ca$_{1.2}$ signaling complexes. Caldendrin adds to the growing list of CaBPs that interact with and regulate voltage-gated Ca$^{2+}$ channels, and should be considered when assessing the heterogeneous properties of these channels in the nervous system.

**Ca$^{2+}$-independent Interactions of CaBPs with Ca$^{2+}$ Channels**—

Binding of Ca$^{2+}$ to the functional EF-hand domains in CaBP1 and caldendrin induce conformational changes that, similar to those observed for CaM, could influence interactions with target molecules (45, 46). However, our findings show that caldendrin does not require Ca$^{2+}$ for binding to α$_{1.2}$ (Figs. 1C, 5C, and 7). The Ca$^{2+}$ independence of caldendrin/Ca$_{1.2}$ interactions are not surprising given that CaBP1 also binds to and regulates Ca$_{1.2}$ and Ca$_{2.1}$ in the absence of Ca$^{2+}$ (15–17). For Ca$_{2.1}$ channels containing the auxiliary β$_{1b}$ subunit, CaBP1 does not affect I$_{Ca}$ inactivation but enhances I$_{Na}$ inactivation (47). Moreover, the related CaBP, visinin-like protein 2, has the opposite effect of slowing I$_{Na}$ inactivation in β$_{1b}$-containing...
Ca<sub>2.2</sub> channels (47). Amino acid substitutions in the second EF-hand prevent CaBP1 and caldendrin from binding Ca<sup>2+</sup> (46), which may allow constitutive association of these proteins with α<sub>1.2</sub> in contrast to the Ca<sup>2+</sup>-dependence of CaM binding (Fig. 1). It should be noted that in the absence of Ca<sup>2+</sup> and Mg<sup>2+</sup>, bound to EF-hand-1 of CaBP1 stabilizes the tertiary structure of the molecule (46). Because Mg<sup>2+</sup> is present at millimolar concentrations in neurons (48), Mg<sup>2+</sup> binding to caldendrin may solidify contacts with Ca<sub>1.2</sub> at resting levels of Ca<sup>2+</sup>.

**Distinct Effects of CaBPs on Ca<sub>1.2</sub> Inactivation**—The diverse actions of CaBPs on Ca<sub>1.2</sub> inactivation may result from binding to distinct sequences in α<sub>1.2</sub> that control this process. In addition to the C-terminal IQ and Pre-IQ sites, the NT of α<sub>1.2</sub> also serves as a critical binding element for CaM and CaBP1 (17, 49). CaM binding to the IQ-domain rather than the NT causes CDI (21–23), while CaBP1 binding to the NT and the IQ-domain of α<sub>1.2</sub> inhibits CDI (16, 17). The inability of caldendrin to bind to the NT (Fig. 1B) may therefore explain why it did not suppress inactivation of I<sub>Ca</sub> as severely as CaBP1 (Figs. 3 and 4). It follows that the moderate inhibition of CDI by caldendrin may result from its interactions strictly with the C-terminal IQ-domain of α<sub>1.2</sub>. The competitive binding of CaM and caldendrin to the IQ-domain (Fig. 2A and C) suggests that caldendrin inhibits CDI by displacing CaM from the IQ-site. That the IQ-EE substitution prevented the direct interaction and modulation of Ca<sub>1.2</sub> by caldendrin (Fig. 5C) further implicates the IQ-domain as a key determinant through which caldendrin exerts its unique effects on Ca<sub>1.2</sub> inactivation. In the crystal structure of the α<sub>1.2</sub> IQ domain in complex with Ca<sup>2+</sup>/CaM, the first isoleucine of the IQ-domain is completely and exclusively buried in hydrophobic contacts with the C-terminal lobe of CaM (41, 43). Due to sequence differences in the C-terminal lobes of caldendrin and CaM (2), similar interactions of this isoleucine residue with caldendrin may not be favored so as to weaken Ca<sup>2+</sup>-dependent mechanisms of inactivation (50, 51). Alternatively, caldendrin may have additional indirect effects on Ca<sub>1.2</sub> inactivation. Previous reports indicate a cytoskeletal interaction of caldendrin but not CaM with microtubule-associated proteins (8). Given that disruption of the cytoskeleton significantly affects CDI of L-type channels (52, 53), caldendrin could destabilize interactions of Ca<sub>1.2</sub> with the cytoskeleton so as to decrease CDI. A potential link of Ca<sub>1.2</sub> channels with the cytoskeleton through caldendrin may further distinguish modulation of Ca<sub>1.2</sub> channels by caldendrin, CaBP1, and CaM.

Given that both CaBP1-S and CaBP1-L are identical to caldendrin over most of their sequence, the differences in how caldendrin and CaBP1 functionally interact with Ca<sub>1.2</sub> are surprising. However, nearly half of the N-terminal sequence of caldendrin is absent in CaBP1-S or CaBP1-L (Fig. 6A). This additional sequence replaces 15 and 75 amino acids in the rat CaBP1-S and CaBP1-L, respectively, including a consensus site for N-terminal myristoylation (Fig. 6A). This unique N-terminal sequence in caldendrin and/or lack of myristoylation could prevent caldendrin from interacting with the NT of α<sub>1.2</sub> and causing slow inactivation of Ca<sub>1.2</sub> I<sub>Ca</sub> like CaBP1. Unlike CaM, which is not myristoylated, caldendrin may modulate Ca<sub>1.2</sub> inactivation only through interactions with the C-terminal domain of α<sub>1.2</sub>, either because it is unable to interact with the NT, or because its interaction with the IQ- and/or Pre-IQ sites prevents the influence of the NT on inactivation. Interestingly, structural analyses indicate that CaBP1 forms a dimer in solution (46). Dimerization of CaBP1 may facilitate intramolecular interactions involving NT and C-terminal domains of α<sub>1.2</sub>, which ultimately suppress I<sub>Ca</sub> inactivation (49, 54). Whether an inability of caldendrin to dimerize distinguishes its effects on Ca<sub>1.2</sub> inactivation from those of CaBP1 awaits further structural analyses.

**Caldendrin as a Modulator of Ca<sup>2+</sup> Signals in Neurons**—The immunofluorescent labeling of Ca<sub>1.2</sub> channels and caldendrin in cultured cortical neurons (Fig. 8) agrees with previous descriptions of postsynaptic distributions of Ca<sub>1.2</sub> channels (44, 55) and caldendrin (8). Our findings that caldendrin colocalizes with Ca<sub>1.2</sub> in some but not all somatodendritic puncta (Fig. 8) suggest the potential for caldendrin to regulate subpopulations of neuronal Ca<sub>1.2</sub> channels. Based on our biochemical evidence that CaM and CaBP1 are not associated with Ca<sub>1.2</sub>/caldendrin complexes in the brain (Fig. 7), we propose that Ca<sub>1.2</sub> channels may differentially associate with CaBPs in particular cellular and subcellular domains. Selective association of Ca<sub>1.2</sub> with CaM, CaBP1, or caldendrin would have distinct functional consequences. Compared with CaBP1, which completely blocks I<sub>Ca</sub> inactivation during repetitive stimuli (16, 17), caldendrin causes a more modest suppression of I<sub>Ca</sub> inactivation (Fig. 4). Therefore, Ca<sub>1.2</sub> Ca<sup>2+</sup> signals in somatodendritic microdomains associated with caldendrin would be intermediate in amplitude compared with those with CaM or CaBP1. Studies of L-type channel inactivation in thalamocortical neurons (56), neocortical pyramidal neurons (57), and hippocampal pyramidal neurons (58) indicate a more moderate level of CDI than that because of endogenous CaM and CaBP1 in recombinant systems (16, 17, 21–23). That caldendrin may contribute to this intermediate level of CDI in some neurons is supported by our electrophysiological, biochemical, and immunocytochemical studies. Considering the diverse effects of caldendrin and other CaBPs as modulators of voltage-gated Ca<sup>2+</sup> channels, defining their significance in regulating neuronal Ca<sup>2+</sup> signals remains an important challenge for future studies.

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