The $\alpha_1$-$\beta$-Subunit Interaction That Modulates Calcium Channel Activity Is Reversible and Requires a Competent $\alpha$-Interaction Domain*

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High voltage-gated calcium channels consist of a pore-forming subunit ($\alpha_1$) and three nonhomologous subunits ($\alpha_2/\delta$, $\beta$, and $\gamma$). Although it is well established that the $\beta$-subunit promotes traffic of channels to the plasma membrane and modifies their activity, the reversible nature of the interaction with the $\alpha_1$-subunit remains controversial. Here, we address this issue by examining the effect of purified $\beta_{2a}$ protein on CaV1.2 and CaV2.3 channels expressed in Xenopus oocytes. The $\beta_{2a}$-subunit binds to the $\alpha_1$-interaction domain (AID) in vitro, and when injected into oocytes, it shifts the voltage dependence of activation and increases charge movement to ionic current coupling of CaV1.2 channels. This increase depended on the integrity of AID but was not abolished by bafilomycin, demonstrating that the $\alpha_1$-$\beta$ interaction through the AID site can take place at the plasma membrane. Furthermore, injection of $\beta_{2a}$ protein inhibited inactivation of CaV2.3 channels and converted fast inactivating CaV2.3/$\beta_{1b}$ channels to slow inactivating channels. Inhibition of inactivation required larger concentration of $\beta_{2a}$ in oocytes expressing CaV2.3/$\beta_{1b}$ channels than expressing CaV2.3 alone but reached the same maximal level as expected for a competitive interaction through a single binding site. Together, our data show that the $\alpha_1$-$\beta$ interaction is reversible in intact cells and defines calcium channels $\beta$-subunits as regulatory proteins rather than stoichiometric subunits.

High voltage-gated calcium channels are multi-subunit protein complexes where a pore-forming subunit combines with one or more nonhomologous auxiliary subunits (1). One of these auxiliary subunit, the $\beta$-subunit, is crucial for channel function, because in addition to stimulating channel activity it appears to be required for surface expression of the channel protein (2). These two effects combined result in a severalfold increase in the ionic current density in heterologous expression and increases charge movement to ionic current coupling of CaV1.2 channels. This increase depended on the integrity of AID but was not abolished by bafilomycin, demonstrating that the $\alpha_1$-$\beta$ interaction through the AID site can take place at the plasma membrane. Furthermore, injection of $\beta_{2a}$ protein inhibited inactivation of CaV2.3 channels and converted fast inactivating CaV2.3/$\beta_{1b}$ channels to slow inactivating channels. Inhibition of inactivation required larger concentration of $\beta_{2a}$ in oocytes expressing CaV2.3/$\beta_{1b}$ channels than expressing CaV2.3 alone but reached the same maximal level as expected for a competitive interaction through a single binding site. Together, our data show that the $\alpha_1$-$\beta$ interaction is reversible in intact cells and defines calcium channels $\beta$-subunits as regulatory proteins rather than stoichiometric subunits.

EXPERIMENTAL PROCEDURES

Protein Preparation, Binding Assay, and Mutagenesis—The cDNA encoding the rat $\beta_{2a}$ subunit (3) was subcloned by conventional PCR methods into pRSET vector (Stratagene) to add an N-terminal polyhistidine tag (His$_6$-$\beta_{2a}$). The His$_6$-$\beta_{2a}$ was expressed in BL-21 (DE-3) Escherichia coli bacteria by 2 h of induction with 0.5 mM isopropyl-$\beta$-D-thiogalactopyranoside at 37 °C and purified from the cleared cell lysate by metal affinity.

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2 The abbreviations used are: AID, $\alpha$-interaction domain; cRNA, complementary RNA; GST, glutathione S-transferase; HA, hemagglutinin; GV, normalized conductance.
chromatography (Talon; BD Biosciences) followed by size exclusion chromatography on a Superdex™ S-200 column (Amersham Biosciences) pre-equilibrated with buffer containing 50 mM Tris buffer, 300 mM NaCl, 1 mM EDTA, pH 8.0. The fractions containing the protein were pooled, concentrated up to 2–4 mg/ml, and stored at −80 °C. Binding to AID was assayed as by Neely et al. (4), using a glutathione-S-transferase (GST) fusion protein encoding the I-I loop of the CaV1.2 subunit (GST-AID), and as a negative control we used GST alone or fused to a 126-amino acid peptide derived from the C-terminal end of the chloride channel from human skeletal muscle ClC-1 (GST-ClC126).

Oocyte Injections and Electrophysiological Recordings—*Xenopus laevis* oocytes were prepared, injected, and maintained as in previous report (4). All capped cRNA were synthesized using the MESSAGEmachine (Ambion, Austin, TX), suspended in 10 μl of water and stored in 2-μl aliquots at −80 °C until use. The CaV1.2 subunit used in this study bears a deletion of 60 amino acids at the N-terminal end that increase expression (10), and the CaV2.3 subunit corresponds to the human form (11). The W470S mutation on the CaV1.2 subunit was incorporated by BAPTA (1,2-bis(2-AMINO-PHENOXO)-ETHANE-N,N,N',N'-TETRAACETIC ACID) in the internal solution for a better control of calcium-activated chloride currents. Data acquisition and analysis were performed using the pCLAMP system and software (Axon Instruments Inc., Foster City, CA). The currents were filtered at 2 kHz and digitized at 10 kHz. Linear components were eliminated by P/−4 prepulse protocol (3). For experiments with the purified β2a protein, the oocytes were injected with 50 nl of the protein stock solution 2–7 h before recordings. The final protein concentration was calculated assuming an oocyte volume of 525 nl that corresponds with a diameter of 1 mm. For bafilomycin treatment, the oocytes were exposed for 24 h to 500 nM concentration obtained from a 1 mM stock solution of bafilomycin A1 (Sigma-Aldrich) in Me2SO and compared with control oocytes incubated for the same period in 0.05% Me2SO.

Surface Expression Measurements in Xenopus Oocytes—Surface expression of CaV1.2 was measured by immunoassay as described (13). The hemagglutinin (HA) epitope was inserted into the extracellular loop S5-H5 of domain II at residue 713 of the CaV1.2 subunit (Swiss-Prot P15381) by standard overlapping PCR using complementary oligonucleotides encoding the HA epitope and flanked with extra amino acids to yield CaV1.2-HA. 5–6 days after CaV1.2 RNA injection, the oocytes were separated into two groups: one for electrophysiological recordings and the other for immunoassay. Unless otherwise stated, all of the incubations for immunoassay were carried out at 4 °C. The oocytes were incubated in ND96 solution (96 mM NaCl, 2 mM KCl, 1.8 mM CaCl2, 1 mM MgCl2, and 5 mM Hepes, pH 7.6) with 1% bovine serum albumin (blocking buffer) for 30 min and then for 60 min in the presence of 1 μg/ml rat monoclonal anti-HA antibody (3F10; Roche Applied Science). After washes, the oocytes were incubated for 30 min with horseradish peroxidase-conjugated secondary antibody (goat anti-rat FAB fragments; Jackson ImmunoResearch). Thereafter, the oocytes were extensively washed with blocking buffer and rinsed once in ND96 at room temperature. Individual oocytes were then placed in 50 μl of SuperSignal enzyme-linked immunosorbent assay fento substrate (Pierce) in 96-well microplates (Optiplate; PerkinElmer Life Sciences), and chemiluminescence was quantified 30 s later with a luminometer (Viktor2; PerkinElmer Life Sciences).

RESULTS

*Purified β2a Protein Binds to GST-AID Fusion Protein and Modulates CaV1.2 Channel Activity—*The purified β2a elutes as a monodisperse peak from a size exclusion chromatography (Fig. 1A) and was capable of binding specifically to the CaV1.2-AID site because no binding activity was observed with GST alone or GST fused to an unrelated sequence (GST-ClC126, Fig. 1B). As in cRNA coexpression experiments (3, 14), the purified β2a protein increases ionic currents and shifts the voltage dependence of calcium channel activation toward more negative potentials. Maximal barium current (Ibar) was increased from −18 ± 4 nA (CaV1.2 alone) to −184 ± 19 nA (CaV1.2/β2a).
protein), which compares with $-101 \pm 12 \text{nA}$ obtained from oocytes expressing Ca$_{V1.2}$(/B$_{2a}$) cRNA (Fig. 1C). The reason for this difference is not clear. Channel expression depends on several factors, such as translation efficiency of microinjected cRNA and protein trafficking capacity of the oocyte, which complicates interpretation of peak current data. Nevertheless, our result shows the functional competence of the B$_{2a}$ protein.

The fraction of channels activated during a 66-ms pulse to increasing potentials was measured as the peak of the tail current during repolarization to $-40 \text{mV}$. Normalized peak tail currents were plotted with respect to the pulse potential to yield the GV curves (Fig. 1D). The sum of two Boltzmann distributions was adjusted to each GV curve, and the parameters defining these distributions are described in Table 1. The only visible difference when comparing the effect of B$_{2a}$ as cRNA or as protein was that with the latter the relative contribution of the first Boltzmann distribution ($G_1$) is slightly smaller (42.0 \pm 2.5\%) than with Ca$_{V1.2}$(/B$_{2a}$ cRNA (50.9 \pm 2.0\%). This change impacts macroscopic conductance at positive voltages and may reflect channels not being modulated by the auxiliary subunit when injected as a protein. To illustrate this point we constructed a GV curve by adding 80% of a GV obtained with Ca$_{V1.2}$(/B$_{2a}$ cRNA and 20% of the one obtained without B-subunit (thick line in Fig. 1D). This new plot superimposes almost perfectly with the data obtained from oocytes injected with the Ca$_{V1.2}$(/B$_{2a}$ protein combination.

Currents recorded in the absence of exogenous B-subunit showed a rapid outward transient corresponding to gating currents that was followed by a small nonactivating inward current mediated by the influx of Ba$^{2+}$ (Fig. 2A, $top$ panel). In contrast, oocytes co-injected with Ca$_{V1.2}$(/B$_{2a}$ cRNA displayed inward ionic currents that were larger than gating currents (Fig. 2A, $middle$ panel). Likewise, injection of the B$_{2a}$ protein to Ca$_{V1.2}$-expressing oocytes also leads to an increase in ionic current amplitude relative to gating currents (Fig. 2A, $bottom$ panel). Here we integrated the first 2 ms of the gating current evoked by a pulse from $-80$ to $-30 \text{mV}$ ($Q_{2ms}$, shaded area in Fig. 2A) and compared it with $I_{Ba}$ measured at the end of a pulse to 0 mV as described previously (15). Although in this type of measurements, $I_{Ba}$/$Q_{2ms}$ may be overestimated because inward currents are subtracted from outward gating currents, the impact is the same for B$_{2a}$ cRNA or B$_{2a}$ protein, because voltage dependence of activation is similar for both subunit combinations.

Fig. 2B shows scatter plots of the $Q_{2ms}$ versus $I_{Ba}$ for the three channel subunit combinations. Over a wide spectrum of $Q_{2ms}$ amplitudes, $I_{Ba}$ amplitudes are near 0 when Ca$_{V1.2}$ was expressed alone, as reflected in an average $I_{Ba}$/$Q_{2ms}$ ratio of $0.3 \pm 0.1 \text{nA/pC}$. When the B$_{2a}$-subunit is present in either form, this ratio is severalfold larger (12.7 \pm 3.9 nA/pC for Ca$_{V1.2}$/B$_{2a}$ protein and 14.2 \pm 2.3 nA/pC for Ca$_{V1.2}$/B$_{2a}$ cRNA). All of the values obtained from Ca$_{V1.2}$/B$_{2a}$ cRNA oocytes fell within the 95\% confidence limit of the regression analysis from Ca$_{V1.2}$/B$_{2a}$ cRNA, indicating that both forms of B$_{2a}$ appear equally effective in facilitating ionic currents at 0 mV.

The Effect of B$_{2a}$ Protein Is Preserved in Ca$_{V1.2}$-expressing Oocytes Pretreated with Bafilomycin but Not When the AID Site Is Disrupted—An increase in ionic currents upon injection of B$_{2a}$ occurs in a few hours, whereas the gating currents remain more or less invariant. This suggests that Ca$_{V1.2}$ subunits already present in the plasma membrane are accessible to the injected B$_{2a}$ protein. To further examine this possibility, we pretreated Ca$_{V1.2}$-expressing oocytes with bafilomycin, an inhibitor of V-type ATPases that impairs the traffic of intracellular vesicles (16). This treatment is expected to interrupt the incorporation of new Ca$_{V1.2}$ subunits into the plasma membrane and cause a net reduction of channel density caused by constitutive endocytosis that removes membrane proteins at a
rather constant rate. To assess that bafilomycin was effectively preventing the incorporation of newly synthesized channels, surface expression was assayed concurrently by two independent methods: immunoassay and charge movement (\(Q_{on}\)) during a voltage step to \(I_{Ba}\) reversal potential (Fig. 3A). Immunoreactivity and \(Q_{on}\) were reduced by more than 70% by bafilomycin, and this reduction was not reverted upon injection of \(\beta_{2a}\) protein. For protein-injected oocytes \(Q_{on}\) was 164 ± 26 pC (n = 8), which compares with 144 ± 21 pC (n = 9) measured in the absence of \(\beta_{2a}\). Similarly, chemiluminescence measurements were virtually identical with \(0.97 \times 10^5 \pm 0.24 \times 10^5\) cps; \(n = 16\) or without \(1.02 \times 10^5 \pm 0.31 \times 10^5\) cps; \(n = 20\) \(\beta_{2a}\) protein. Thus, in fact the bafilomycin treatment under our conditions prevents channel incorporation into plasma membrane.

If \(\beta_{2a}\) could only interact with channels before they reach the plasma membrane, then injection of \(\beta_{2a}\) protein should not modify the function of calcium channels in bafilomycin-treated oocytes. However, \(I_{Ba}/Q_{on}\) increases from 0.6 ± 0.2 nA/pC to 18.6 ± 5.6 nA/pC by injection of \(\beta_{2a}\) protein in bafilomycin-treated oocytes (Fig. 3, B and C), indicating that \(\beta_{2a}\) interacts with channels already present in the plasma membrane.

We next asked whether modulation of function of mature channels depends on an intact AID site and generated a point mutation abolishes modulation of function as well as binding (18). Because expression may be impaired by alteration of the AID sequence, we documented the ability of HA-tagged CaV1.2-W470S to reach the plasma membrane, then injection of CaV1.2-HA cRNA alone, after injection with \(\beta_{2a}\) protein, treated with 500 nM bafilomycin for 24 h, or injected with \(\beta_{2a}\) protein after bafilomycin treatment (\(CaV1.2/\beta_{2a}\) prot. + Baf). C, scatter plot of \(Q_{on}\) versus \(I_{Ba}\) from oocytes injected with \(CaV1.2\) cRNA in control conditions (\(CaV1.2\)), treated with 500 nM bafilomycin for 24 h (\(CaV1.2+\text{Baf}\)), or injected with \(\beta_{2a}\) protein after bafilomycin treatment (\(CaV1.2/\beta_{2a}\) prot. + Baf). D, scatter plot of \(Q_{on}\) versus \(I_{Ba}\) from oocytes expressing CaV1.2-W470S alone or injected with \(\beta_{2a}\) protein 3–5 h before recording. E, scatter plot of \(Q_{on}\) versus \(I_{Ba}\) from oocytes expressing CaV1.2-W470S alone (\(\text{WT}\)) or following injection of \(\beta_{2a}\) protein (\(\text{WT}\), F, average current versus voltage plot. Peak current amplitudes (at +20 mV) were -39 ± 4 nA for W470S (n = 19) (C) and -38 ± 7 nA for W470S/\(\beta_{2a}\) prot. (n = 12) (C). WT, wild type.

We also show that substituting the conserved tryptophan within the AID sequence in CaV1.2 abolishes binding of \(\beta_{2a}\) protein to a peptide carrying this mutation (Fig. 4C, GST-AIDW470S). When injected into CaV1.2-W470S-expressing
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FIGURE 5. Inhibition of inactivation of CaV2.3 and CaV2.3/β2a-mediated Iα by β2a protein. A, representative current traces during a 10 s pulse to 0 mV from a holding potential of -90 mV from oocytes expressing CaV2.3 alone and CaV2.3 and CaV2.3/β2a, following injection of β2a protein. B, bar plots of T1/2 for the different subunit combinations. C, ionic current traces during steady state inactivation protocol from CaV2.3 and CaV2.3/β2a expressing oocytes a few hours following the injection of β2a protein. The steady state inactivation protocol consisted of a 10-s prepulse followed by a 0.5-s test pulse to 0 mV. The prepulse was increased in 15-mV steps and repeated every 60 s. D, average steady state inactivation plot for CaV2.3 expressing (n = 9, C) and CaV2.3/β2a expressing (n = 16, ○) oocytes injected with β2a protein. Boltzmann distributions that best described each set of data are shown as continuous lines (see Table 2 for details). For comparison, templates curves CaV2.3SSin (dotted line) and CaV2.3/β2aSSin (dashed line) from Fig. 7 are also included.

TABLE 2
Mean ± S.E. of parameters defining the Boltzmann distribution and the percentage of residual current (IRES) that best fitted steady state inactivation for the different subunit combinations

Peak current (IPEAK) during the test pulse (Fig. 5, C and D) were plotted against the prepulse potential (V) and adjusted to the following equation: IPEAK / IRES = V - V1/2 + (IPEAK / IRES) / (1 + exp((V1/2 - V) / Z)). For comparison, templates curves were obtained by adjusting Bolzmann distributions to discriminate among both models, we studied the inhibition of inactivation by measuring T1/2 at different concentrations of β2a (Fig. 6). In CaV2.3/β1b channel complexes, increase in T1/2 requires higher concentration of β1b than CaV2.3 alone (1.2 μM versus 0.24 μM to increase T1/2 near half-maximal, respectively).

| Subunit composition | n | V1/2 | Z | IRES |
|---------------------|---|------|---|------|
| CaV2.3              | 11 | -51.9 ± 1.2 | 2.20 ± 0.11 | 0.5 ± 0.9 |
| CaV2.3/β2a-prot.    | 9  | -43.5 ± 1.0  | 2.20 ± 0.14 | 28.2 ± 3.5 |
| CaV2.3/β2a-cRNA     | 10 | -40.9 ± 4.6  | 1.89 ± 0.13 | 17.2 ± 2.7 |
| CaV2.3/β1b-cRNA     | 10 | -70.3 ± 1.1  | 2.34 ± 0.17 | 0.1 ± 0.5 |
| CaV2.3/β1b-cRNA + β2a | 18 | -61.4 ± 2.0  | 1.48 ± 0.06 | 10.5 ± 1.4 |

increases from 0.26 ± 0.03 s (n = 16) for CaV2.3 alone to 4.0 ± 0.63 s (n = 12). This is even slower than T1/2 values obtained from oocytes injected with both subunits as cRNAs (2.4 ± 0.42 s; n = 13). More notably, the β2a protein was also capable of increasing T1/2 in oocytes coexpressing the CaV2.3/β1b combination, from 0.15 ± 0.01 s (n = 18) to 1.5 ± 0.3 s (n = 23). The steady state inactivation (SSin) is also affected by β2a-subunit combination (20). While SSin is complete for CaV2.3 alone or coexpressed with β1b, a residual component emerges (IRES) with β2a. When we injected β2a protein in oocytes expressing CaV2.3 alone or the CaV2.3/β1b combination, voltage for half-inactivation was shifted to the right, and a significant component of IRES developed in both cases (Fig. 5, C and D; see also Table 2 for details).

The inhibition of inactivation by β2a protein in CaV2.3/β1b channel complexes may arise either by competitive inhibition or by allosteric modulation through a second binding site. To discriminate among both models, we studied the inhibition of inactivation by measuring T1/2 at different concentrations of β2a (Fig. 6). In CaV2.3/β1b channel complexes, increase in T1/2 requires higher concentration of β1b than CaV2.3 alone (1.2 μM versus 0.24 μM to increase T1/2 near half-maximal, respectively).

With higher β2a concentration (>3 μM) channels inactivate similarly regardless of the presence of β1b (T1/2 = 6.4 ± 1.5 s (n = 4) and 6.8 ± 2.0 s (n = 5) for CaV2.3 alone or coexpressed with β1b, respectively). These results suggest a simple competitive replacement of β1b by β2a.

To estimate the fraction of newly associated CaV2.3/β2a channel complexes, we modeled the voltage dependence of steady state inactivation of CaV2.3 and CaV2.3/β1b channels exposed to different concentrations of β2a protein (Fig. 7). Each subunit combination is expected to give rise to a SSt in of a particular shape. In oocytes expressing a mixture of subunit combinations, SSt in corresponds to the weighted sum of the template curves characterizing inactivation of CaV2.3, CaV2.3/β1b, and CaV2.3/β2a channels (CaV2.3SSin, CaV2.3/β1bSSin, and CaV2.3/β2aSSin, respectively; Fig. 7A, inset). The three template curves were obtained by adjusting Boltzmann distributions to average IPEAK / IRES curves from oocytes with the different subunit combinations. Following this approach we determined the relative weight of the CaV2.3/β2a channel complexes (% β2a-like SSin) and plotted against the different concentrations of β2a protein tested. From these plots, we obtained a dissociation constant of 0.35 μM for β2a on CaV2.3-expressing oocytes and 0.85 μM in CaV2.3/β1b background without changes in the max-
imal effect. Concomitantly, the relative weight of CaV2.3/β1b channels (%β1b-like SSIN) decreases with increasing concentration of β2a protein when β1b is present (Fig. 7B). Note also that in oocytes lacking the β1b-subunit, this component of inactivation was not detected. Together these results confirm the notion of a competitive reaction between β1b and β2a.

**DISCUSSION**

The data presented here show that the α1-β-subunit interaction leading to modulation of channel activity can take place at the plasma membrane, is reversible, and depends on an intact AID site. The reversibility was shown by a novel approach that took advantage of the differential effect of two β-subunit isoforms, β1b and β2a, on the inactivation of CaV2.3 channels. Through a simple modeling of the voltage dependence of the steady state inactivation of the different subunit combinations, we estimated the $K_D$ in intact cells. CaV2.3/β1b channels were converted to channels with a behavior typical of CaV2.3/β2a channels upon the addition of β2a protein in a concentration-dependent manner that was consistent with the exchange of β-subunits governed by mass action law; i.e. the presence of β1b shifts the apparent $K_D$ without a change in the maximal effect when compared with CaV2.3 alone. Thus, our data are consistent with a competitive inhibition of inactivation of CaV2.3/β1b channel complexes by β2a protein. Although our analysis does not conclusively exclude a second binding site, evidence for a single site comes from experiments showing that a covalently linked β2a to CaV1.2 recapitulates the effect of the auxiliary subunit on expression and function and no longer responds to coexpression of the β-subunit (9). In our case, with both subunit combinations, dose-response curves yield Hill coefficients between one and two (Fig. 7). Several factors may increase the apparent Hill coefficient, such as not attaining full equilibrium in the 5 h that elapsed between protein injection and electrophysiological recordings. According to our experience, longer incubation time reduces the activity of the purified β-subunit protein (4).

The $K_D$ values we measured in intact cells are hundred-fold larger than reported for in vitro binding to GST-AID fusion proteins. Affinity for the β-subunit may be lower for channels in the cell surface than in early stage of protein synthesis and folding as initially proposed by Birnbuamer and co-workers (2). A $K_D$ value in the micromolar range is also consistent with a rapidly reversible binding of the β-subunit that stands in contrast to the traditional view of an invariant stoichiometric subunit. This reversibility is important to other intracellular regulatory pathways involving the AID site. For example, G protein binds to a site near the AID sequence, displaces the β-subunit, and inhibits N and P/Q type calcium channel (21, 22).

An issue that remains rather puzzling is our observation that CaV1.2-W470S, a mutant with impaired β-subunit binding and functional modulation, expresses well in the plasma membrane, as if binding of the β-subunit to the AID site were not mandatory for surface expression. It may be that the lack of the conserved tryptophan weakens binding to the AID to the point that is no longer detectable in vitro and on functional modulation, but it is sufficient to allow the β-subunit to occlude the putative endoplasmic reticulum retention site encoded in the I–II loop. In light of recent experiments (23) showing that CaV2.1 can also reach the plasma membrane without the AID sequence, retention appears not to be as tight as initially thought, and perhaps the β-subunit may help traffic through the endoplasmic reticulum by binding to other sites of the α1-subunit. Alternatively, the CaV1.2 N-terminal truncation used in this study may help override the retention signal from the AID conferring independence of channel expression on the
β-subunit. We have reported similar results for other β-subunit isoforms (4). This uncoupling would also explain why N-terminal deletion mutant overexpresses (10).

In summary, we demonstrate that the interaction between the β-subunit and the pore-forming subunit of voltage-gated calcium channels is dynamic. The dynamic nature of this interaction allows for post-targeting modulation of channel function. Moreover, this interaction requires the AID site, proposed to control targeting of the channel to the plasma membrane.

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