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

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Patricia Hidalgo1‡, Giovanni Gonzalez-Gutierrez‡, Jennie Garcia-Olivares‡, and Alan Neely†1

From the 1Abteilung Neurophysiologie, Medizinische Hochschule Hannover, 30625 Hannover, Germany, the 2Centro de Estudios Científicos, Valdivia 509000, Chile, and the 3Centro de Neurociencia de Valparaíso, Universidad de Valparaíso 2349400 Valparaíso, Chile

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 subunits, 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 systems, but the relative contribution, biological relevance, and extent to which both processes are independent from each other remain elusive. Early studies show that in Xenopus oocytes, coexpression of $\beta_{2a}$ with the pore-forming $\alpha_1$ subunit from cardiac cells (CaV1.2) augments ionic currents mostly by increasing ionic current to charge movement ratio (3). Later, it was shown that the addition of the $\beta$-subunit as purified protein is capable of modulating channel activity of the $\alpha_1$ subunit expressed in Xenopus oocytes (4, 5) and also on isolated membranes from skeletal muscle (6). These results suggest that modulation of function is separated from the effect on channel expression and predicts that binding sites remain available on the mature channel. However, the $\alpha_1$-$\beta$-subunit association depends primarily on the so-called $\alpha$-interaction domain (AID),2 located within the intracellular loop joining the first and second repeats of the $\alpha_1$-subunit. Secondary binding sites have been identified, but they appear to be specific to certain $\alpha_1$-$\beta$ pairs, and they modulate particular aspects of channel function (7, 8). Recent work indicates that binding of a single $\beta$-subunit recapitulates function (9). Hence, it seems likely that $\beta$-subunit-binding sites available at the plasma membrane arise from unbinding of the $\beta$-subunit to the AID site during channel trafficking. This would imply a reversible interaction rather than a stoichiometric association, as proposed by Tareilus et al. (2). Here, we purified the $\beta_{2a}$-subunit isof orm, injected in oocytes expressing $\alpha_1$-subunit, and measured ionic and gating currents. Gating currents were correlated to the number of channels in the membrane by immunobassay. Using this approach we demonstrate that the $\alpha_1$-$\beta$-subunit interaction is dynamic and can occur at the plasma membrane. Binding to mature channels is reversible, and association of the $\beta$-subunit to the AID site is an absolute requirement to modulate channel function.

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
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-II loop of the CaV1.2 subunit (GST-AID), and as a negative control we used GST alone or fused to a 126-aminophenyl acid peptide derived from the C-terminal end of the chloride channel from human skeletal muscle CIC-1 (GST-CIC126).

**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 MESSAGE-machine (Ambion, Austin, TX), resuspended 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 CaV3.2 subunit corresponds to the human form (11). The W4705 mutation on the CaV1.2 subunit was incorporated by standard PCR methods. Electrophysiological recordings using the cut-open oocyte technique (12) with a CA-1B amplifier (Dagan Corp., Minneapolis, MN) were performed 4–6 days after cRNA injection as described (4). The external solution contained in mM, 10 Ba2+, 96 n-methylglucamine, and 10 HEPES, pH 7.0, and the internal solution 120 n-methylglucamine, 10 EGTA, and 10 HEPES, pH 7.0. For recording of oocytes expressing the CaV3.2 subunit, EGTA was replaced by BAPTA (1,2-bis(o-aminophenoxy)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 normalized by Coomassie Brilliant Blue staining.

**Surface Expression Measurements in Xenopus Oocytes**—Surface expression of CaV1.2 was measured by immunoassay as described (13). The hemagglutinin (HA) epitope was inserted as a negative control we used GST alone or fused to a 126-aminophenyl acid peptide derived from the C-terminal end of the chloride channel from human skeletal muscle CIC-1 (GST-CIC126).

**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-CIC126; 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 (I_{\text{BA}}) was increased from −18 ± 4 nA (CaV1.2 alone) to −184 ± 19 nA (CaV1.2/β2a) 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-coupled 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 femto substrate (Pierce) in 96-well microplates (Opti-plate; PerkinElmer Life Sciences), and chemiluminescence was quantified 30 s later with a luminometer (Viktor2; PerkinElmer Life Sciences).

**Reversible α1-β Interaction in Calcium Channels**

![Figure 1](image-url)

**FIGURE 1. Functional assays of the β2a protein.** A, size exclusion chromatography profile of β2a, B, in vitro binding assay of β2a to GST-AID. The binding reaction started when the β2a protein was added to GST, GST-CIC126, or GST-AID fusion protein coupled to glutathione-Sepharose beads. After the binding reaction was completed, proteins bound to the resin were eluted with SDS loading buffer, resolved on SDS-PAGE (10% acrylamide gel), and visualized by Coomassie Brilliant Blue staining. Lane 1, molecular mass standard (kDa); lane 2, β2a; lane 3, GST-AID; lane 4, binding to GST-AID; lane 5, GST; lane 6, binding to GST; lane 7, GST-CIC126; lane 8, binding to GST-CIC126. C, average current versus voltage plot for the different subunit combinations. Peak current amplitudes were −18 ± 4 nA at +20 mV for CaV1.2 cRNA, −101 ± 12 nA for CaV1.2/β2a cRNA, and −184 ± 19 nA for CaV1.2-cRNA/β2a protein (C) at +10 mV. D, average normalized conductance versus voltage plot for the different subunit combinations. The continuous lines correspond to the fit of the sum of two Boltzmann distributions as in Table 1. The thick line describes the sum of 0.8 times the GV obtained with CaV1.2/β2a cRNA and 0.2 times the one obtained without the β-subunit (see Table 1 for details).
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TABLE 1
The mean ± S.E. of parameters defining the sum of two Boltzmann distributions that best fitted the normalized conductance

GV values were obtained by measuring the peak of tail currents recorded during deactivation at $-40$ mV following depolarizing pulses of increasing voltages from a holding potential of $-80$ mV. The sum of two Boltzmann distributions was adjusted to tail currents amplitudes from individual experiments. $\%G_1$ is the relative contribution of component developing at more negative potential. Each Boltzmann distribution is characterized by slope factors $z_1$ and $z_2$ and half activation potential $V_1$ and $V_2$ as used by Olcese et al. (24). $I_{na}$ corresponds to the average maximum tail current estimated from the fit.

<table>
<thead>
<tr>
<th>CaV1.2 (n = 9)</th>
<th>CaV1.2/β2a cRNA (n = 8)</th>
<th>CaV1.2/β2a protein (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$%G_1$</td>
<td>55.4 ± 6.6</td>
<td>50.9 ± 2.0</td>
</tr>
<tr>
<td>V1 (mV)</td>
<td>62.3 ± 2.7</td>
<td>10.0 ± 1.5</td>
</tr>
<tr>
<td>V2 (mV)</td>
<td>2.09 ± 0.06</td>
<td>2.59 ± 0.13</td>
</tr>
<tr>
<td>V3 (mV)</td>
<td>95.3 ± 4.4</td>
<td>57.0 ± 1.7</td>
</tr>
<tr>
<td>$z_2$</td>
<td>1.44 ± 0.12</td>
<td>1.18 ± 0.03</td>
</tr>
<tr>
<td>$I_{max}$ (μA)</td>
<td>-2.87 ± 0.50</td>
<td>-0.76 ± 0.08</td>
</tr>
</tbody>
</table>

FIGURE 2. Gating and $Ba^{2+}$ currents from oocytes expressing CaV1.2-cRNA alone or combined with $\beta_{2a}$ cRNA or $\beta_{2a}$ protein. A, representative gating and ionic current traces from oocytes injected with CaV1.2-cRNA (CaV1.2), CaV1.2/β2a-cRNA (CaV1.2/β2a cRNA), and CaV1.2/β2a protein (CaV1.2/β2a prot) during 60-ms voltage pulses to −30, 0, and +30 mV from a holding potential of −80 mV. The horizontal bars signal 30 ms for the pulse to −30 mV displayed as inset on an expanded scale. The initial 2 ms under the outward transients, corresponding to gating currents, was integrated to obtain $Q_{2ms}$ (highlighted as the gray shaded area). The vertical bars in the inset correspond to 10 nA. B, scatter plot of $Q_{2ms}$ versus the current amplitude ($I_{na}$) at the end of the pulse to 0 mV from oocytes injected with CaV1.2-cRNA alone (n = 28, A), CaV1.2/β2a-cRNA (n = 13, O), and CaV1.2/β2a protein (n = 27, C). Continuous and dashed lines correspond to ±95% confidence limits for the regression analysis of CaV1.2/β2a-cRNA and CaV1.2-cRNA expressing oocytes, respectively.

The effect of $\beta_{2a}$ protein is preserved in CaV1.2-expressing oocytes pretreated with bafilomycin but not when the AID site is disrupted. An increase in ionic currents upon injection of $\beta_{2a}$ occurs in a few hours, whereas the gating currents remain more or less invariant. This suggests that CaV1.2 subunits already present in the plasma membrane are accessible to the injected $\beta_{2a}$ protein. To further examine this possibility, we pretreated CaV1.2-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 CaV1.2 subunits into the plasma membrane and cause a net reduction of channel density caused by constitutive endocytosis that removes membrane proteins at a

protein), which compares with $-101 ± 12$ nA obtained from oocytes expressing CaV1.2/β2a 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 β2a 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 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 β2a as cRNA or as protein was that with the latter the relative contribution of the first Boltzmann distribution (G1) is slightly smaller (42.0 ± 2.5%) than with CaV1.2/β2a cRNA (50.9 ± 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 CaV1.2/β2a cRNA and 20% of the one obtained without β-subunit (thick line in Fig. 1D). This new plot superimposes almost perfectly with the data obtained from oocytes injected with the CaV1.2/β2a protein combination.

Currents recorded in the absence of exogenous β-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 coinjected with CaV1.2/β2a cRNA displayed inward ionic currents that were larger than gating currents (Fig. 2A, middle panel). Likewise, injection of the $\beta_{2a}$ protein to CaV1.2-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 mV ($Q_{2ms}$ shaded area in Fig. 2A) and compared it with $I_{na}$ measured at the end of a pulse to 0 mV as described previously (15). Although in this type of measurements, $Q_{2ms}$ may be overestimated because inward currents are subtracted from outward gating currents, the impact is the same for $\beta_{2a}$ cRNA or $\beta_{2a}$ protein, because voltage dependence of activation is similar for both subunit combinations.

Fig. 2B shows scatter plots of the $Q_{2ms}$ versus $I_{na}$ for the three channel subunit combinations. Over a wide spectrum of $Q_{2ms}$ amplitudes, $I_{na}$ amplitudes are near 0 when CaV1.2 was expressed alone, as reflected in an average $I_{na}/Q_{2ms}$ ratio of 0.3 ± 0.1 nA/pC. When the β2a-subunit is present in either form, this ratio is severalfold larger (12.7 ± 3.9 nA/pC for CaV1.2/β2a protein and 14.2 ± 2.3 nA/pC for CaV1.2/β2a cRNA). All of the values obtained from CaV1.2/β2a protein oocytes fell within the 95% confidence limit of the regression analysis from CaV1.2/β2a cRNA and CaV1.2-cRNA expressing oocytes, indicating that both forms of β2a appear equally effective in facilitating ionic currents at 0 mV.

The effect of $\beta_{2a}$ protein is preserved in CaV1.2-expressing oocytes pretreated with bafilomycin but not when the AID site is disrupted. An increase in ionic currents upon injection of $\beta_{2a}$ occurs in a few hours, whereas the gating currents remain more or less invariant. This suggests that CaV1.2 subunits already present in the plasma membrane are accessible to the injected $\beta_{2a}$ protein. To further examine this possibility, we pretreated CaV1.2-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 CaV1.2 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: immunosassay and charge movement (Q_{on}) during a voltage step to I_{Ba} reversal potential as shown in the inset. Voltage near I_{Ba} reversal potential was determined empirically by stepping to several potential in 2-mV increments. B, gating and ionic current traces (I_{Ba}) recorded as in Fig. 2, from oocytes expressing CaV_{1.2} alone in control conditions (CaV_{1.2}), treated with 500 nm bafilomycin for 24 h (CaV_{1.2}+Baf.), or injected with β_{2a} protein after bafilomycin treatment (CaV_{1.2}+β_{2a} prot.+ Baf.). C, scatter plot of Q_{2ms} versus I_{Ba} from oocytes injected with CaV_{1.2} cRNA in control conditions (○), with bafilomycin treatment (●) and bafilomycin-treated oocytes injected with β_{2a} protein (▲).}

18.6 ± 5.6 nA/pC by injection of β_{2a} protein in bafilomycin-treated oocytes (Fig. 3, B and C), indicating that β_{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 in CaV_{1.2} (W470S) homologous to a mutation that impairs binding of the β-subunit in CaV_{2.1} (17). In CaV_{2.3}, this 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 CaV_{1.2} carrying the W470S mutation in CaV_{1.2} (W470S-HA) cRNA (W4705-HA), and noninjected oocytes. B, Q_{2ms} versus chemiluminescence for oocytes expressing CaV_{1.2}-HA and CaV_{1.2}-W4705-HA. The continuous lines correspond to the linear regression, whereas the dashed lines correspond to the ±95% confidence limit of the regression analysis. C, binding of β_{2a} protein to GST-AID and GST-AIDW470S. The binding reaction was carried out as in Fig. 1. Lane 1, molecular mass in kDa; lane 2, β_{2a} protein; lane 3, GST-AID fusion protein; lane 4, binding to GST-AID; lane 5, GST, lane 6, binding to GST; lane 7, GST-AIDW4705; lane 8, binding to GST-AIDW4705. The gating and ionic current traces (I_{Ba}) were recorded from oocytes expressing CaV_{1.2}-W4705 alone or injected with β_{2a} protein 3–5 h before recording. E, scatter plot of Q_{2ms} versus I_{Ba} from oocytes expressing CaV_{1.2}-W4705 alone (○) or following injection of β_{2a} protein (▲). F, average current versus voltage plot. Peak current amplitudes (at +20 mV) were -39 ± 4 nA for W4705 (n = 19, ○) and -38 ± 7 nA for W4705/β_{2a} prot. (n = 12, ▲). WT, wild type.
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![Figure 5](image_url)

**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

<table>
<thead>
<tr>
<th>Subunit composition</th>
<th>n</th>
<th>V½</th>
<th>Z</th>
<th>IRES %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca2.3</td>
<td>11</td>
<td>−51.9 ± 1.2</td>
<td>2.20 ± 0.11</td>
<td>0.5 ± 0.9</td>
</tr>
<tr>
<td>Ca2.3/β2a-prot.</td>
<td>9</td>
<td>−43.5 ± 1.0</td>
<td>2.20 ± 0.14</td>
<td>28.2 ± 3.5</td>
</tr>
<tr>
<td>Ca2.3/β2a-cRNA</td>
<td>10</td>
<td>−40.9 ± 4.6</td>
<td>1.89 ± 0.13</td>
<td>17.2 ± 2.7</td>
</tr>
<tr>
<td>Ca2.3/β1b-cRNA</td>
<td>10</td>
<td>−70.3 ± 1.1</td>
<td>2.34 ± 0.17</td>
<td>0.1 ± 0.5</td>
</tr>
<tr>
<td>Ca2.3/β1b-cRNA + β2a</td>
<td>18</td>
<td>−61.4 ± 2.0</td>
<td>1.48 ± 0.06</td>
<td>10.5 ± 1.4</td>
</tr>
</tbody>
</table>

Increases from 0.26 ± 0.03 s (n = 16) for Ca2.3 alone to 4.0 ± 0.63 s (n = 12). This is even slower than T½ values obtained from oocytes injected with both subunits as CRAs (2.4 ± 0.42 s; n = 13). More notably, the β2a protein was also capable of increasing T½ in oocytes coexpressing the Ca2.3/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 (20). While SSIN is complete for Ca2.3 alone or coexpressed with β1b, a residual component emerges (IRES) with β2a. When we injected β2a protein in oocytes expressing Ca2.3 alone or the Ca2.3/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 Ca2.3/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 T½ at different concentrations of β2a (Fig. 6). In Ca2.3/β1b channel complexes, increase in T½ requires higher concentration of β2a than Ca2.3 alone (1.2 μM versus 0.24 μM to increase T½ near half-maximal, respectively). With higher β2a concentration (>3 μM) channels inactivate similarly regardless of the presence of β1b (T½ = 6.4 ± 1.5 s (n = 4) and 6.8 ± 2.0 s (n = 5) for Ca2.3 alone or coexpressed with β1b, respectively). These results suggest a simple competitive replacement of β1b by β2a.

To estimate the fraction of newly associated Ca2.3/β2a channel complexes, we modeled the voltage dependence of steady state inactivation of Ca2.3 and Ca2.3/β1b channels exposed to different concentrations of β2a protein (Fig. 7). Each subunit combination is expected to give rise to a SSIN of a particular shape. In oocytes expressing a mixture of subunit combinations, SSIN corresponds to the weighted sum of the template curves characterizing inactivation of Ca2.3, Ca2.3/β1b, and Ca2.3/β2a channels (Ca2.3/SSIN, Ca2.3β1bSSIN, and Ca2.3β2aSSIN, respectively; Fig. 7A, inset). The three template curves were obtained by adjusting Boltzmann distributions to average IPEAK/IMAX curves from oocytes with the different subunit combinations. Following this approach we determined the relative weight of the Ca2.3/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 Ca2.3-expressing oocytes and 0.85 μM in Ca2.3/β1b background without changes in the max-
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FIGURE 6. Dose dependence of β_{2a}-induced inhibition of inactivation in CaV_{2.3} and CaV_{2.3}/β_{1b} channels. Bar plots of T_{1/2} at different concentrations of β_{2a} protein in CaV_{2.3}-expressing (light gray) and CaV_{2.3}/β_{1b}-expressing (dark gray) oocytes.

FIGURE 7. Dose dependence of β_{2a-like} SSIN in CaV_{2.3} and CaV_{2.3}/β_{1b} expressing oocytes. A, plot of the average weight of CaV_{2.3}/β_{1b} SSIN expressed as percentage (% β_{2a-like} SSIN) versus the concentration of β_{2a} protein in oocytes injected with CaV_{2.3} cRNA alone (○) or CaV_{2.3}/β_{1b} cRNA (●). B, PEAK/MAX curves from individual oocytes were adjusted to the weighted sum of CaV_{2.3SSIN}, CaV_{2.3}/β_{1b}SSIN, and CaV_{2.3}/β_{1b}/β_{2a}SSIN (inset). Each dose-response curve was then adjusted to β_{2a-like}SSIN = 1/(1+(I/K_{D}^\text{PEAK/\text{MAX}})) for CaV_{2.3}-expressing oocytes K_{D}^\text{PEAK/\text{MAX}} = 0.35 μM and n = 1.5 and for CaV_{2.3}/β_{1b}-expressing oocytes K_{D}^\text{PEAK/\text{MAX}} = 0.85 μM and n = 1.7. B, percentage of β_{2a-like} SSIN from the same analysis shown in A. The error bars are within the symbols for CaV_{2.3} expressing oocytes. The continuous line corresponds to 1−β_{2a-like} SSIN.

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, β_{1b} and β_{2a}, on the inactivation of CaV_{2.3} 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. CaV_{2.3}/β_{1b} channels were converted to channels with a behavior typical of CaV_{2.3}/β_{2a} channels upon the addition of β_{2a} 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 β_{1b} shifts the apparent K_{D} without a change in the maximal effect. Concomitantly, the relative weight of CaV_{2.3}/β_{1b} channels (% β_{1b-like} SSIN) decreases with increasing concentration of β_{2a} protein when β_{1b} is present (Fig. 7B). Note also that in oocytes lacking the β_{1b}-subunit, this component of inactivation was not detected. Together these results confirm the notion of a competitive reaction between β_{1b} and β_{2a}.

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 CaV_{1.2-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 was 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 signal encoded in the I–II loop. In light of recent experiments (23) showing that CaV_{2.1} 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 CaV_{1.2} N-terminal truncation used in this study may help override the retention signal from the AID conferring independence of channel expression on the...
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$\beta$-subunit. We have reported similar results for other $\beta$-subunit isoforms (4). This uncoupling would also explain why N-terminal deletion mutant overexpresses (10).

In summary, we demonstrate that the interaction between the $\beta$-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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REFERENCES