New Short Splice Variants of the Human Cardiac Caβ2 Subunit

REDEFINING THE MAJOR FUNCTIONAL MOTIFS IMPLEMENTED IN MODULATION OF THE Ca1.2 CHANNEL*

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Two new short splice variants of the Ca2+ channel β2 subunit were cloned from human heart poly(A)(+)+ mRNA. The 410-amino acid β2α subunit is encoded by exons 1A, 2A, 3, 4, 12, 13, and 14 of the human Caβ gene and lacks the protein kinase A phosphorylation site, the β-interaction domain (De Waard, M., Pragnell, M., and Campbell, K. P. (1994) Neuron 13, 495–505), 40% of the β-SH3 domain, and 73% of the guanylate kinase domain of the putative membrane-associated guanylate kinases module (McGee, A. W., Nunziato, D. A., Maltez, J. M., Prehoda, K. E., Pitt, G. S., and Bredt, D. S. (2004) Neuron 42, 89–99), and helix α3 of the α1-subunit binding pocket (Van Petegem F., Clark, K. A., Chatelain, F. C., and Minor, D. L., Jr. (2004) Nature 429, 671–675). The β2α transcript has two potential initiation codons. With the second ATG codon, it generates the 164-amino acid β2αβ subunit essentially by the distal part of exon 14, and thus β2αβ completely lacks any of the above motifs. Immunoprecipitation analysis confirmed stable association of β2α and β2αβ with the α1C subunit. The plasma membrane localization of β2α and β2αβ increased by co-expression of the α1C,77 and α1Cδ subunits. In COS1 cells, β2α and β2αβ increased plasma membrane targeting of the pore-forming α1C subunit and differentially facilitated (β2α > β2αβ) the voltage gating of otherwise silent Ca1.2 channels. We conclude that it is unlikely that the β-interaction domain, membrane-associated guanylate kinases module, and the α1-subunit binding pocket helices α5 are essential for the interaction of the α1C and β subunits and suggest that in addition to the α1-subunit binding pocket helices α5 and α8, a yet unresolved C-terminal β2 region plays a crucial role.

The L-type high voltage-activated Ca1.2 channel is composed of the pore-forming α1C subunit and the auxiliary β and α1Cδ subunits. The Caβ subunits (1) are essential cytoplasmic modulators of the Ca2+ channel activity that generate a molecular signal necessary for the facilitation of voltage gating as well as for the correct plasma membrane targeting of the functional Ca1.2 complex (2). The α1C and β subunits are tightly associated in the channel complex and can be co-immunoprecipitated in mild non-ionic detergents. A conserved β-interaction domain (BID) common for genetically different β subunits (β1–β4) was proposed as a binding motif interacting with the conserved α-interaction domain (AID) of the I-II cytoplasmic linker between repeats I and II of an α1 subunit (3, 4). The α1–β interaction is believed to chaperon the channel by inhibiting an endoplasmic reticulum retention signal encoded in the α1 subunit I-II linker (5). Comparative studies showed that β subunits differentially modulate inactivation kinetics (6) and single-channel properties of the Ca1.2 channel (7). We have found that the differential β-subunit modulation predominantly influences the slow inactivation of the channel and is associated with distinct voltage-gated rearrangements between the N-terminal regions of the α1C and β2 subunits (8). Structural principles underlying the β-subunit modulation of Ca2+ channels were also approached by a search of structural homology with known regulatory proteins. It has been found (9) that the vast central conservative region of β2 subunits shares distant homology with the Srg homology 3 (SH3)-guanylate kinase (GK) module of membrane-associated guanylate kinases (MAGUKs). This hypothesis was further elaborated by studying mutations of the rat (N terminus-palmitoylated) β2α subunit that interfere with interactions between SH3 and GK and affect inactivation of Ba2+ currents (10, 11). Intramolecular interactions between the β-subunit SH3 and GK homologues were supported by the results of the recent high-resolution crystallography studies of the β-subunit “cores” of SH3-GK domains alone or in complex with AID (12–14). These studies also showed that BID is not available for the binding to AID because it is buried inside the β-subunit structure. Instead, the α-subunit binding pocket (ABP) distal to the SH3 domain was inferred as a structure engaged in the interaction with AID.

The first cardiac β2 subunits cloned from rat (15) and rabbit (16) hearts have over time been co-identified with five N-terminal splice variants (β2α–β2αβ; for overview, see Ref. 17). A recent comprehensive study revealed that in the human left ventricle there are nine Caβ2 splice variants, including β2αβ, β2αβ, β2α, and β2αβ: the exon 7C isoforms of β2αβ, β2αβ, and β2αβ the exon 7B isoform of β2αβ as well as the β2αβ transcript lacking exon 7 and truncated in the exon 8 region (18). Here, we report on two new splice variants of the Caβ2 channel β2 subunit gene cloned from the human normal heart poly(A)(+) mRNA and sequentially named β2αβ and β2αβ. The β2α and β2αβ transcripts lack exons encoding a single protein kinase A (PKA) phospho-

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† The abbreviations used are: BID, β-interaction domain; AID, α-interaction domain; SH3, Src homology 3; GK, guanylate kinase domain; MAGUK, membrane-associated guanylate kinase; ABP, α-subunit binding pocket; PKA, protein kinase A; RT, reverse transcriptase; nt, nucleotide(s); EYFP, enhanced yellow fluorescent protein; ECFP, enhanced green fluorescent protein; αα, amino acid(s); HEK, human embryonic kidney; GFP, green fluorescent protein; β2αβ, rabbit cardiac β2 subunit; Vα, holding potential.
rylation site, BID, as well as a large part (β2) or the entire SH3-GK module (β2g). Despite the wide structural differences between β2f/β2g and the rest of the β subunits, the new β subunits yielded fully functional Ca1.2 channels with different inactivation characteristics when co-expressed in COS1 cells with the αδ and the human vascular α1C,δ subunits. These data indicate the need to redefine the significance of the previously outlined β subunit functional motifs and implicate the C-terminal region encoded by the distal β2 gene exon in modulation of the channel.

MATERIALS AND METHODS

Cloning of the Short Human Cardiac Ca1.2αβ2 Subunits—The Ca2+ channel β2f and β2g subunits were cloned from the human heart poly(A)+ mRNA pooled from three Caucasian males (ages 35, 55, and 55; cause of death, trauma) (BD Biosciences Clontech, Palo Alto, CA; catalog number 65533-1) using C. therm. Polymerase One-Step reverse transcriptase (RT)-PCR (Roche Applied Science). The RT reaction was carried out for 30 min at 57 °C with a common RT oligonucleotide primer, 5′-ACATATGATTGCAGTGTAGACC-3′, and the sense primer 5′-GCTGTTAGTTATACAAGACTTC-3′ (nt 605181–605201) primer, respectively. PCR mixtures were denatured for 2 min at 94 °C followed by 30 PCR cycles, each composed of denaturing for 45 s at 94 °C, annealing for 1 min at 51 °C, and extension for 2.5 min at 72 °C, with the last step of the last cycle extended to 10 min. For the second round of PCR with “nested primers,” a common antisense 5′-gcttagagTCATTGGCCGGAATGAACTC-3′ primer (nt 803958– 803978, attached to an XbaI linker shown in small letters) was used with the sense 5′-gccaccATGTTGAGGAGGATCCTGAG-3′ (β2f) and 5′-gccaccATGTTGAGGAGGATCCTGAG-3′ (β2g) primers for the clone of chromosome 10. The RT-PCR products were purified using the QiAquick PCR purification kit (Qiagen, Valencia, CA), ligated into the pcCR2.1 TA vector (Invitrogen) and sequenced in both directions. To generate β2g, the β2g-coding TA clone was amplified using an antisense primer, as shown above for the second round of PCR, and the sense primer 5′-gccaccATGTTGAGGAGGATCCTGAG-3′ (β2f) primer (incorporated into the small letters in the ATG start codon) and the same PCR conditions except with an increased annealing temperature (56 °C). The RT-PCR products were purified using the QiAquick PCR purification kit (Qiagen, Valencia, CA). Se-quence analysis was performed with the Genetic Analytical software (Compix, Pittsburgh, PA).

RESULTS

Molecular Cloning and Structural Properties of the Short Human Cardiac Ca1.2αβ2 Subunits—Two new short splice variants of the Ca2+ channel β2 subunit gene, named β2f and β2g (Fig. 1A), were cloned by RT-PCR from the poly(A)+ fraction of the human normal heart mRNA. The RT and PCR primers for the cloning were deduced from the Human Genome Project draft sequence NT_008705.15 of chromosome 10. The β2f subunit has a theoretical molecular mass of 45.8 kDa, is composed of 410 aa, and has the N terminal encoded by exons 1A and 2A analogous to β2g (18). However, missing in the β2f transcript are exons 5–11 coding for the 250-aa central region of the β2f protein, including BID, as well as 55 of 136 aa (i.e. 40%) of the C-terminal part of the SH3 domain and 192 of 262 aa (i.e. 73%) of the N-terminal part of GK domain. Overall, the following crystallographically resolved structures (13) are absent from the β2f protein: antiparallel β-strands β1–β5 and the second α-helix (α2) of the first conserved domain, the entire variable domain V2, as well as parallel β-sheets β6–β9, two α-helices (α3–α4) and two β1 helices (n2–n3) of the second conserved domain. Thus, the entire linker between SH3 and GK (“HOO domain”) present in MAGUKs is deleted from the β2f subunit.

The even smaller β2g-subunit transcript is composed of exon 2e followed by the 72-nt upstream portion of exon 3 and the 463-nt distal part of exon 14. Although exon 2e encodes the N terminus of the β2g subunit, the reading frame is interrupted just 150 nt downstream of the first initiation codon. The second ATG codon occurs 91 nt downstream from the first one, or 34 nt downstream from the acceptor splice site of exon 3. Its reading frame includes the distal 463-nt sequence of the β2g 3′-terminal exon 14. Thus, both exons 3 and 14 are alternatively spliced in β2g, but the respective utilized donor and acceptor splice sites do not conform to the consensus sequences. With the second initiation codon, the β2g-subunit transcript encodes a 164-aa protein with the calculated molecular mass of 19.5 kDa. This shorter variant, referred further as β2g2, has been generated by a PCR deletion of the 91-nt upstream region including the first ATG codon. The β2g subunit lacks the PKA phosphorylation site (RKST in position 216 of the human β2g) that was shown to be involved in the PKA-mediated stimulation of cardiac L-type Ca2+ currents (20). In addition, β2g retains only 6 of 12 potential protein kinase C phosphorylation sites, (S/T)(R/K), in positions 78, 198, 302, 343, 382, and 441.

The last three sites are also present in the β2g subunit.

Although β2f and β2g were cloned from the polyadenylated fraction of mRNA, none of the studied short β subunit splice variants has yet been shown to be expressed in the human heart as functional proteins. Because of this and as the usage of initia-
tion codons in β2g remains unknown, β2Mg is considered a putative splice variant of the β2g subunit. Nevertheless, because β2Mg lacks not only BID and the entire SH3-GK domains but also the variable β2-subunit N termini-coding exons, β2Mg is particularly interesting for the study of the functional significance of these genetically deleted motifs. To investigate whether the structural deletions discovered in the new β2 splice variants affect their interaction with the α1C subunits, co-immunoprecipitation of the short β2 subunits with the wild-type α1C,77 was analyzed by Western blot assay (Fig. 1B). ECFP was genetically fused to the N termini of the new β2 splice variants by in-frame ligation of the ECFP and β2/β2Mg coding sequences, and the (ECFP)Nβ2 variants and α1C,77 from the solubilized membrane particulate fraction of HEK293 cells helped to avoid a contamination with the nonassociated cytosolic (ECFP)Nβ2 and the membrane-bound orphan α1C subunits. Both β2 (Fig. 1B, lane 1) and β2Mg (lane 2) pulled down the α1C,77 protein from the solubilized membrane preparations suggesting stable association between the subunits. A similar observation was made with β1α1 and β2α1 (8), and other β proteins. In controls, no immunoprecipitation of the α1C subunit by anti-GFP antibody was found in the absence of (ECFP)Nβ2 variants in COS1 cell with (Fig. 1B, lane 3) or without (lane 4) EGFP co-expressed. These data indicate that the α2δ and α1C subunits are necessary for the plasma membrane targeting by the β2α1 and β2Mg subunits.

The β2 Subunit Stimulates the Plasma Membrane Targeting and Facilitates the Ca\textsubscript{v}1.2 Channel Voltage Gating—To better characterize the cellular location of the expressed α1C and β subunits, the (ECFP)Nβ2-F and β2Mg were expressed with α1C,77 and α2δ subunits in COS1 cells lacking the endogenous Ca\textsuperscript{2+} channels (21). In contrast to the data obtained with Xenopus oocytes (10, 14) and HEK293 cells (11), heterologous expression of the α1C and α2δ subunits in COS1 cells did not induce an appreciable Ca\textsuperscript{2+} channel activity (Fig. 2A) unless a β subunit was co-expressed (Fig. 2B). Thus, selection of the COS1 cells expression system allowed us to avoid ambiguity in the assessment of the β-subunit modulation of the Ca\textsuperscript{2+} channel typical for the cited studies (10, 11, 14) and to define the β-subunit modulation here as a facilitation of the Ca\textsuperscript{2+} channel voltage gating by a β subunit. Essential prerequisites for a β-subunit modulation of the channel are binding of Ca, β to the α1C subunit and targeting of the oligomeric channel complex to the plasma membrane.

In the absence of Ca, β, the (EYFP)N-β2 subunit is essentially retained in intracellular compartments of the cell (Fig. 2C, a). Similar to β2α1 (Fig. 2E, c) (8), co-expression of the β2f subunit increased surface membrane targeting of (EYFP)N-α1C,77, which is evident from a comparison of the subcellular

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**FIG. 1. Cloning and co-immunoprecipitation analysis of the β2f and β2g subunits.** A. A schematic map of the human cardiac β2 subunit transcripts including all exons. The β2f and β2g transcripts are compared with β2a. Exons (boxed) are numbered according to Foell et al. (18), and their size (in base pairs) is shown below each exon. ATG and TGA above the exons indicate initiation and termination codons, respectively. An approximate position of BID (bold horizontal line) and SH3 and GK motifs (lines with double arrowhead) is illustrated above the β2a transcript. The spliced-out exons in the β2f and β2g transcripts and their parts in exons 3 and 14 of the β2a transcript are shown as gray boxes (unnumbered). Note that the β2f transcript is initiated from exon 2c similar to β2a. Because the reading frame of the β2g transcript opened at the first initiation codon is interrupted at the position 153, the second ATG (shown above exon 3) at the position 98 was utilized to generate the β2Mg transcript. B. Co-immunoprecipitation with the short β2 splice variants. The (ECFP)Nβ2f (1), (ECFP)Nβ2g (2) or no β subunit (3,4) was co-expressed in HEK293 cells with (1-3) or without (4) the α1C,77 and α2δ subunits. EGFP was co-expressed in control sample 3. Cells were lysed by freeze-thaw. To remove free β subunits and other cytosolic proteins, the crude membrane particulate fractions were prepared from the lysed cells as described earlier (8). Membranes were solubilized with 0.5% Nonidet P-40, and the channel proteins were immunoprecipitated with the antibody against green fluorescent protein, separated by SDS-polyacrylamide gel electrophoresis, and analyzed by immunoblotting. The (ECFP)N-labeled β2f splice variants (1,2) and EGFP (3) were detected on the blot by the monoclonal antibody against green fluorescent protein, whereas the co-immunoprecipitated α1C subunit was detected using the anti-α1C antibody.
distribution of fluorescence in the absence (Fig. 2C, a) and in the presence of β_{2f} (Fig. 2C, b). Conversely, α_{1C} increased plasma membrane targeting of the β_{2f} subunit. In the absence of α_{1C}, the (ECFP)$_{N}$-labeled β_{2f} subunit was diffusely distributed over the cytoplasm (Fig. 2C, c). A similar intracellular localization, typical for cytosolic proteins, was observed with the full-size (ECFP)$_{N}$-β_{2(r)} (Fig. 2E, a). Co-expression of α_{1C,77} increased the plasma membrane localization of the (ECFP)$_{N}$-β_{2f} subunit (Fig. 2C, d) analogously to the effect of α_{1C,77} on the (ECFP)$_{N}$-β_{2(r)}, in positive control (Fig. 2E, b). These data corroborate the results of immunoprecipitation analysis (Fig. 1B, lane 1) and provide strong evidence that β_{2f} binds to α_{1C,77} and facilitates the channel formation and its plasma membrane targeting. Thus, the β_{2f} subunit displayed both the chaperon function and binding to α_{1C}, which are features characteristic for β_{2(r)} and other β subunits (2).

**Fig. 2.** Effect of the β_{2f} subunit on functional expression, subcellular distribution, and electrophysiological properties of the Ca$^{2+}$ channel. A, $I_{Na}$ recorded from a COS1 cell transfected by the (EYFP)$_{N}$-α_{1C,77} and α$_{2}$δ subunits (inset shows the whole-cell EYFP) in response to a 600-ms test pulse to +20 mV from the holding potential of −90 mV. B, panel a, representative traces of $I_{Na}$ in response to the indicated test pulses (V$_{h}$ = −90 mV) from a COS1 cell transiently transfected with the (EYFP)$_{N}$-α_{1C,77}, α$_{2}$δ and β$_{2f}$ subunits. Panel b, phase-contrast cell image with a shadow of patch pipette; panel c, whole-cell EYFP fluorescence. Panel d, average normalized $I$-$V$ relationship (n = 9) for $I_{Na}$ through the (EYFP)$_{N}$-α_{1C,77}/α$_{2}$δ/β$_{2f}$ channel. C, whole-cell fluorescence from COS1 cells expressing the (EYFP)$_{N}$-α_{1C,77} and α$_{2}$δ subunits in the absence (a) or in the presence (b) of the β$_{2f}$ subunit or from COS1 cells expressing the (ECFP)$_{N}$-β$_{2r}$ and α$_{2}$δ subunits in the absence (c) or in the presence (d) of the α_{1C,77} subunit. The expression level of the labeled proteins was approximately the same (compare unadjusted fluorescence scales in the right panels). D, representative traces of $I_{Ca}$ (a) or $I_{Ba}$ (b–d) evoked by a test pulse to +20 mV applied for 0.6 (a, b), 5 (c), or 30 s (d). E, whole-cell fluorescence from COS1 cells expressing the (ECFP)$_{N}$-β$_{2(r)}$ subunit in the absence (a) or in the presence (b) of the α_{1C,77} and α$_{2}$δ subunits. Control panel c shows surface membrane targeting of (EYFP)$_{N}$-α_{1C,77} co-expressed with the α$_{2}$δ and β$_{2(r)}$ subunits. Panel d, representative 30-s trace of $I_{Ba}$ through the (EYFP)$_{N}$-α_{1C,77}/α$_{2}$δ/β$_{2(r)}$ channel (8). V$_{h}$ = −90 mV. Dashed lines show a zero current. Scaling bars, 3 μm.
Despite the large structural differences between \( \beta_{2f} \) and the other known \( \beta_2 \) subunits, when co-expressed in COS1 cells with the human vascular \( \alpha_{1C,77} \) subunit and \( \alpha_2 \), the new \( \beta_{2f} \) subunit yields a fully functional Ca\( \text{II} \),1.2 channel. Fig. 2B (panel c) shows representative traces of the Ba\( \text{II} \) current (\( I_{\text{Ba}} \)) evoked by the 600-ms test pulses to the indicated voltages applied from \( V_h = -90 \) mV. The corresponding averaged normalized I-V relationship is presented in Fig. 2B, d. The values for the half-maximal activation (\( V_{0.5} = 1.8 \pm 1.7 \) mV) and the slope factor (\( k_{I-V} = -7.9 \pm 0.9 ; n = 9 \)) were essentially similar to those reported earlier for \( I_{\text{Ba}} \) through the \( \alpha_{1C,77}/\alpha_2/\beta_{2f} \) channel expressed in Xenopus oocytes (22). Respectively, a replacement of Ba\( \text{II} \) for Ca\( \text{II} \) as the charge carrier (Fig. 2D, a) produced a typical strong acceleration of the current decay characteristic for Ca\( \text{II} \)-induced inactivation of the Ca\( \text{II} \),1.2 channel. Comparing with the rabbit \( \beta_{2f} \), subunit, the new short \( \beta_2 \) renders notably faster inactivation of \( I_{\text{Ba}} \). Fig. 2D (b–d) shows maximum \( I_{\text{Ba}} \) elicited by test pulses of different durations to \( +20 \) mV. A two-exponential fitting of the 30-s \( I_{\text{Ba}} \) through the (EYPF)N•-\( \alpha_{1C,77}/\alpha_2/\beta_{2f} \) channel (\( n = 5 \)) showed the inactivation time constants (\( \tau \)) and fractions (\( f \)) of the fast and slow components to be \( \tau_f = 465 \pm 20 \) ms, \( f = 64.4 \pm 1.4\% \), and \( \tau_s = 4.31 \pm 0.68 \) ms, \( f = 35.2 \pm 2.1\% \), respectively. Although complete inactivation of the current was not reached even with the 45-s pulse, the fraction of the current that remains by the end of the 30-s pulse (18.0 \pm 2.0\%) was, on average, significantly (\( p < 0.05 \)) smaller than those we have found (27.5 \pm 3.3\%) with the \( \beta_{2f} \) subunit. The fast component of \( I_{\text{Ba}} \) through the (EYPF)N•-\( \alpha_{1C,77}/\alpha_2/\beta_{2f} \) channel (Fig. 2E, d) (\( \tau_f = 499 \pm 68 \) ms; \( I_f = 53.4 \pm 1.6\%; n = 5 \)) was essentially similar to those of the \( \beta_{2f} \) channel. However, the slow component showed a delayed decay (\( \tau_s = 12.88 \pm 2.40 \) s; \( I_s = 19.4 \pm 4.7\%; p < 0.01 \)). Overall, our study revealed that \( \beta_{2f} \) exhibits properties characteristic of the \( \beta_2 \) subunits and yields a fully functional Ca\( \text{II} \),1.2 channel. These data suggest that a requirement of the \( \beta_2 \) subunit for functional expression of the channel (6, 23) is conserved in the regions of \( \beta_2 \) other than BID and the missing essential parts of SH3-GK.

The \( \beta_{2f} \) Subunit Narrows the Functional Correlates of the \( \beta \) Subunit to the C-terminal 153-Amino Acid Region—The immunoprecipitation analysis (Fig. 1B, lane 2) showed that the \( \beta_{2f} \) subunit binds to \( \alpha_{1C,77} \) despite the lack of BID, the entire MAGUK module, and the ABP. The \( \beta_{2f} \) subunit is composed of 164 aa of which the N-terminal 11 residues are new to \( \beta_2 \) subunits. The last 153 aa are common for the C-terminal region of \( \beta_2 \) subunits encoded by the distal part of exon 14. Our data indicate that this C-terminal region is sufficient to confer the assembly of the functional channel. Fig. 3 shows cellular distribution and functional expression of the Ca\( \text{II} \) channel assembled with \( \beta_{2f} \). When co-expressed with \( \alpha_2 \delta \) but in the absence of \( \alpha_{1C} \), the (ECPF)N•-\( \beta_{2f} \) subunit was diffusely distributed over the cytoplasm without selectively targeting the plasma membrane (Fig. 3A, a). The \( \alpha_{1C,77} \) subunit strongly enhanced accumulation of (ECPF)N•-\( \beta_{2f} \) in the plasma membrane (Fig. 3A, panel b). Conversely, \( \beta_{2f} \) effectively increased membrane targeting of the (ECPF)N•-labeled \( \alpha_{1C,77} \) (Fig. 3A, compare c and d). Thus, \( \beta_{2f} \) retains the molecular signals necessary for binding to the \( \alpha_{1C} \) subunit and plasma membrane targeting of the channel.

Co-expression of the \( \alpha_{1C,77}, \alpha_2 \delta, \) and \( \beta_{2f} \) subunits gave rise to a functional Ca\( \text{II} \) channel that exhibited somewhat unusual current properties. First, with both Ca\( \text{II} \) and Ba\( \text{II} \) as the charge carrier, the maximum amplitude of the current was \( \sim 10 \) times smaller than through the \( \alpha_{1C,77}/\alpha_2/\beta_{2f} \) channel. Fig. 3B shows the 600-ms traces of the Ca\( \text{II} \) and Ba\( \text{II} \) currents through the \( \beta_{2f} \) channel evoked by depolarization to \( +20 \) mV from \( V_h = -90 \) mV. Both the activation and inactivation properties of the \( \beta_{2f} \) channel are significantly different from those of the \( \beta_{2f} \) channel. The Ca\( \text{II} \) current did not show accelerated inactivation (Fig. 3B, left trace), which would be characteristic for the Ca\( \text{II} \)-conducting L-type channels (e.g. compare traces a and b in Fig. 2D), thus suggesting that Ca\( \text{II} \)-induced inactivation may be impaired by the \( \beta_{2f} \) subunit. The activation of the Ba\( \text{II} \) current is delayed for \( \sim 1 \) s (Fig. 3, B and C). A two-exponential fitting of inactivation kinetics of the 30-s \( I_{\text{Ba}} \) through the (EYPF)N•-\( \alpha_{1C,77}/\alpha_2/\beta_{2f} \) channel (\( n = 6 \)) showed a substantial decrease of the fast component (\( \tau = 350 \pm 150 \) ms; \( I_f = 16.5 \pm 4.0\% \)), a prolongation of the slow decay (\( \tau_s = 13.9 \pm 5.3 \) s; \( I_s = 47.5 \pm 3.0\% \)), and an almost 2-fold increase of the sustained current component by the end of the 30-s pulse (36.0 \pm 5.5\%). Thus, the \( \beta_{2f} \) and \( \beta_{2f} \) channels clearly exhibit differential modulation of inactivation despite the lack of MAGUK and BID structures. Overall, \( \beta_{2f} \) demonstrates typical properties of the Ca\( \text{II} \) channel \( \beta \) subunits, including binding to the \( \alpha_{1C} \) subunit and stimulation of the surface membrane targeting by the channel, but provides an altered and weak facilitation of channel gating.

**DISCUSSION**

The most interesting result of this study is that vast deletions in the central region of the \( \beta_2 \) subunit that include most of or the entire SH3-GK region do not compromise the \( \beta_2 \)-subunit modulation of the Ca\( \text{II} \),1.2 channel. The \( \beta_2 \) subunit exhibits an array of properties that resemble those of the \( \beta_{1C,77} \) and other \( \beta_2 \) subunits, whereas the shortest known \( \beta_{2f} \) isoform shows...
more unusual features in modulation of the channel. Our results with the β2 subunit confirm the main structural implications of the crystallographic study of the Ca,β2C-AID complex (12–14) by highlighting the fact that it is the C-terminal region encoded by exons 12–14 that appears to bear essential structural bases for ABP or MAGUK, and β2Cg does not share a substantial (>30%) homology with the other β subunits. Experimentally established conventional modulation of the channel by the β2 subunit suggests that the combination of α5 and α8 helices is an important structural requirement for ABP and/or that helix α3 might be replaced in ABP by an unidentified motif(s) in β2C. In contrast, β2xa lacks any structural bases for ABP or MAGUK, and β2Mg does not share a substantial (>30%) homology with the β1, β3, and β4 subunits. Nevertheless, β2Mg binds to the α1C-α7 subunit (Fig. 1B, lane 2), stimulates membrane targeting of the channel (Fig. 3), and shows properties of a weak modulator of the Ca,β2 channel voltage gating. Specifically, β2Mg supports slowly inactivating Ba2+ currents at prolonged depolarization, which is a characteristic feature of other β2 subunits. There are no structural data available for the β2-ABP C-terminal region to explain the results of the functional evaluation of the β2Mg, except to suggest that within the C-terminal 153-amino acid sequence, an essential structure of the β2 subunit is present that endows a modulation of the channel via functional interaction with α1C.

The β subunit is an important component of a molecular complex that determines cytoplasmic helical packing stabilizing channel gating. We hypothesize that it is the Ca,β2 N-terminal half that finely tunes the gating facilitation and affects differential modulation of the Ca2+ channel current inactivation and the voltage-gated rearrangements of the α1C and β subunit N-tails (8). The MAGUK module may contribute to this regulation, but its partial or even complete deletion does not impede the channel modulation as defined in this paper.

Several Ca,β variants with truncated C-terminals were previously identified (18). Our work has established the fact that the main splice isoforms (β2a–β2g) of the β2 subunit gene may generate smaller functional variants through the genetically encoded deletions of the central regions. These data interject significant complications in the reassessment of tissue and cellular distribution of Ca,β subunit variants. Development of specific immunohistochemical tools for β2 and β2g is problematic because they share the same aa sequences with other β2 subunits. Given the large size of untranslated regions of the β2 subunit transcripts (15), relatively small structural deletions encoded in β2a and β2g may be difficult to assess by Northern blot analysis.

Our results also show that alternative splicing of the β2 subunit gene may affect regulation of the Ca,1.2 channels in the human heart by PKA. Activation of PKA through the β-adrenergic receptor pathway is crucial for an up-regulation of the cardiac L-type Ca2+ currents (27). This effect was found to be in part because of PKA phosphorylation of a single specific site of the β2 subunit (20). Because this site is genetically deleted from the naturally occurring short splice variants of the human β2 subunit described in this paper, these variants may have a distinct role (or no role) in human cardiac electrophysiology. In any case, the discovery of the new functional short β2-subunit isoforms lacking many of the predicted functional motifs adds understanding to the molecular bases for the β-subunit modulation of Ca2+ channels and may give rise to new molecular tools of the study of mechanisms of Ca2+ signal transduction.

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REFERENCES
1. Ruth, P., Rohrkaefer, A., Biel, M., Bosse, K., Regulla, S., Meyer, H. E., Flockerzi, V., and Hofmann, F. (1998) Science 245, 1115–1118
2. Dolphin, A. C. (2003) J. Bioenerg. Biomembr. 35, 599–620
3. Pragnell, M., De-Waard, M., Mori, Y., Tanabe, S., Snutch, T. P., and Campbell, R. P. (1984) Nature 308, 67–70
4. De Waard, M., Pragnell, M., and Campbell, K. P. (1994) Neuron 13, 495–503
5. Bichet, D., Cornet, V., Gheb, S., Carlier, E., Velsen, S., Hoshi, T., Mori, Y., and De Waard, M. (2000) Neuron 25, 177–190
6. Lory, P., Varadi, G., Slish, D. F., Varadi, M., and Schwartz, A. (1993) FEBS Lett. 315, 167–172
7. Hullin, R., Khan, I. F. Y., Wirtz, S., Mohacsi, P., Varadi, G., Schwartz, A., and Herzig, S. (2003) J. Biol. Chem. 278, 21623–21630
8. Kobrinsky, E., Kepppling, K. J. F., Yu, A., Harry, J. B., Kahr, H., Romanin, C., Abernethy, D. V., and Soldanor, N. M. (2004) Biophys. J. 87, 844–857
9. Hanlon, M. R., Berrow, N. S., Dolphin, A. C., and Wallace, B. A. (1999) FEBS Lett. 445, 366–370
10. McGee, A. W., Nunziato, D. A., Maltez, J. M., Prehoda, K. E., Pitt, G. S., and Bredd, D. S. (2004) Neuro Report 15, 89–90
11. Takahashi, S. X., Miriyala, J., and Colecraft, H. M. (2004) Proc. Natl. Acad. Sci. U. S. A. 101, 7193–7198
12. Opatowsky, Y., Chen, C.-C., Campbell, K. P., and Hirsch, J. A. (2004) Neuron 42, 387–399
13. Van Petegem, F., Clark, K. A., Chatelain, P. C., and Minor, D. L., Jr. (2004) Nature 429, 671–675
14. Chen, Y. H., Li, M. H., Zhang, Y., He, L.-L., Yamada, V., Fritzmaurice, A., Shen, Y., Zhang, H., Tong, L., and Yang, J. (2004) Nature 429, 675–680
15. Perez-Reyes, E., Castellano, A., Kim, H. S., Bertrand, P., Baggstrom, E., Lacerda, A. E., Wei, X., and Birnbaumer, L. (1992) J. Biol. Chem. 267, 1792–1797
16. Hullin, R., Singer-Lahat, D., Freichel, M., Briel, M., Dascal, N., Hofmann, F., and Flockerzi, V. (1992) EMBO J. 11, 885–890
17. Colecraft, H. M., Alseikhan, B., Takahashi, S. X., Chaudhuri, D., Mittleman, S., Yegnasubramanian, V., Alvania, R. S., Johns, D. C., Marban, E., and Yue, D. T. (2002) J. Physiol. (Lond.) 541, 435–452
18. Foe, J. D., Balijepalli, R. C., Desile, B. P., Yunker, A. M. R., Rohia, S. L., Walker, J. W., McEnery, M. W., January, C. T., and Kamp, T. J. (2004) Physiol. Genomics 17, 183–200
19. Kobriniski, E., Schwartz, E., Abernethy, D. V., and Soldanor, N. M. (2003) J. Biol. Chem. 278, 5021–5028
20. Bunemann, M., Gerhardstein, B. L., Gao, T., and Hosey, M. (1999) J. Biol. Chem. 274, 33851–33854
21. Meir, A., Bell, D. C., Stephens, G. J., Page, K. M., and Dolphin, A. C. (2000) Biophys. J. 79, 731–746
22. Soldanor, N. M., Zühlke, R. D., Bouron, A., and Reuter, H. (1997) J. Biol. Chem. 272, 3560–3566
23. Nishimura, S., Takeshima, H., Hofmann, F., Flockerzi, V., and Imoto, K. (1993) FEBS Lett. 324, 283–286
24. Powers, P. A., Liu, S., Hogan, K., and Gregg, R. G. (1992) J. Biol. Chem. 267, 22967–22972
25. Murakami, M., Wissenbach, U., and Flockerzi, V. (1996) Eur. J. Biochem. 236, 138–143
26. Taviaux, S., Williams, M. E., Harpod, M. M., Nargeot, J., and Lory, P. (1997) Hum. Genet. 100, 151–154
27. McDonald, T. F., Pelzer, S., Trautwein, W., and Pelzer, D. J. (1994) Physiol. Rev. 74, 365–507
New Short Splice Variants of the Human Cardiac Ca\textsubscript{v}β\textsubscript{2} Subunit: REDEFINING THE MAJOR FUNCTIONAL MOTIFS IMPLEMENTED IN MODULATION OF THE Cav1.2 CHANNEL

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