Novel splice variants of the α1 subunit of the Cavα1.2 voltage-gated Ca\(^{2+}\) channel were identified that predicted two truncated forms of the α1 subunit comprising domains I and II generated by alternative splicing in the intracellular loop region linking domains II and III. In rabbit heart splice variant 1 (RH-1), exon 19 was deleted, which resulted in a reading frameshift of exon 20 with a premature termination codon and a novel 19-amino acid carboxyl-terminal tail. In the RH-2 variant, exons 17 and 18 were deleted, leading to a reading frameshift of exons 19 and 20 with a premature stop codon and a novel 62-amino acid carboxyl-terminal tail. RNase protection assays with RH-1 and RH-2 cRNA probes confirmed the expression in cardiac and neuronal tissue but not skeletal muscle. The deduced amino acid sequence from full-length cDNAs encoding the two variants predicted polypeptides of 99.0 and 99.2 kDa, which constituted domains I and II of the α1 subunit of the Cavα1.2 channel. Antipeptide antibodies directed to sequences in the second intracellular loop between domains II and III identified the 240-kDa Cav1.2 subunit in sarcolemmal and ond intracellular loop between domains II and III; and Career Investigator of the Heart and Stroke Foundation of Ontario.

The voltage-gated ion channels determine membrane excitability and regulate signal transduction (1–5). These ion channels consist of multimeric complexes comprising a central α subunit, which contains the structural determinants for ion selectivity, conductance, and voltage sensing, and several auxiliary subunits, which confer regulation on the α subunits (6, 7). The α subunits of K\(^{+}\), Na\(^{+}\), and Ca\(^{2+}\) channels show overall structural similarity in that the α subunit of K\(^{+}\) channels consists of a single domain, which is predicted to contain six membrane-spanning (S1–S6)\(^{1}\) regions, whereas the structural unit of Na\(^{+}\) and Ca\(^{2+}\) channels consists of four such homologous domains (1). Because the K\(^{+}\) channel genes encode only one domain, it is proposed that homo- and heterotetrameric co-assembly of single-domain K\(^{+}\) channel subunits is required to constitute the four-domain channel complexes (8–10).

The different pore-forming α subunits are encoded by distinct gene families (68). Ten genes encoding the voltage-gated Ca\(^{2+}\) channels have been identified; seven (denoted Cavα1.1–1.4 and Cavα2.1–2.3) encode the high voltage-activated channels (5, 11–17), and three (Cavα3.1–3.3) encode the low voltage-activated channels (18–21). Genes encoding different α subunits of the voltage-gated Ca\(^{2+}\) channels exhibit distinct channel properties, which are determined by subtle changes in amino acid composition and appear to be expressed in a tissue- and cell-specific manner (22–28). Alternative splicing, posttranslational modifications, and modulation by auxiliary subunits can generate further diversity in Ca\(^{2+}\) channel heterogeneity, although the four-domain structure is maintained (29–36). For example, alternative splicing of mutually exclusive exons encoding the S3 segment of domain IV of the α1 subunit of Cavα1.2 channels serves as a developmentally regulated switch in cardiac tissue coinciding with major changes in excitation (29). Variability in the carboxyl-terminal region of the α1 subunit of Cavα1.2 channels generated by alternative splicing influences the kinetics as well as Ca\(^{2+}\) and voltage dependence of the L-type Ca\(^{2+}\) channels (32, 33). Moreover, dihydropyridine sensitivity of cardiac and vascular α1 subunits of Cavα1.2 Ca\(^{2+}\) channels may be attributed to tissue-specific expression of an alternatively spliced S6 segment of domain I of the Cavα1.2 gene (36).

Changes in amino acid composition in the extramembrane regions of the α1 subunit appear to have given rise to specialized physiological roles for the different classes of α1 subunits of the Cav\(^{2+}\) channel family (37–41). For example, the structure of the intracellular loop connecting domains II and III appears to be a critical determinant of the mode of signal transmission in the Cavα1.1 and Cavα1.2 α1 subunits (42–44), because this structure of the skeletal (Cavα1.1) but not cardiac (Cavα1.2) α1 subunits can directly interact with the Ca\(^{2+}\) release channel of the sarcoplasmic reticulum to determine the contractile characteristics of skeletal muscle (45–47). Furthermore, loop II–III of Cavα2.1 (P/Q-type) and Cavα2.2 (N-type) Ca\(^{2+}\) channels associates with syntaxin and synaptosomal associated protein 25 to regulate excitation-secretion coupling (50–56).

Because the α1 subunit of Cavα1.2 Ca\(^{2+}\) channels is expressed in tissues such as the myocardium and brain, which are composed of a heterogeneous population of cells, it has been pos-
tulated that alternatively spliced variants may be expressed to serve functions in a cell type-specific manner. In view of the functional importance of the loop II–III structure, we examined whether alternative splicing will result in the generation and expression of structural variants in this critical region. We report here the identification of two splice variants of Cav1.2 Ca\(^{2+}\) channels that generate truncated forms of the \(\alpha_1\) subunits, which were predicted to constitute domains I and II with unique carboxyl-terminal tails. The expression of these variants in cardiac and neuronal tissue suggests that further diversity in Ca\(^{2+}\) channel function and regulation may be generated through alternative structural units.

**EXPERIMENTAL PROCEDURES**

Isolation of RNA and RT-PCR Analysis—Total RNA was isolated using the TriPure reagent (Roche Molecular Biochemicals) according to method described by Chomczynski (48). Adult rabbit heart, brain, and skeletal muscle were frozen in liquid nitrogen, and total RNA was extracted in phenol-chloroform. Total RNA (1.0 \(\mu\)g) was reverse transcribed with either a gene-specific primer, based on the sequence of the \(\alpha_1\) subunit of the Cav1.2 gene from rabbit myocardium described by Mikami et al. (Ref. 17; primer JW6, GGGTGAAGTCGTCGTCGGAC, nt 2536–2561) or primer loop 3 (CCCTCCTTCTCCTGCTTGGCCTCCTC-3', nt 3199–3179) and SuperScript (Invitrogen), and cycle sequenced (PerkinElmer Life Sciences), the rare messages of the Cav1.2 transcript was sequenced. RT-PCR was performed using the Taq DNA polymerase (PerkinElmer). The primer sequences for the expression of the Cav1.2 loop II–III variants, the intensities of protected fragments were size normalized and expressed as a ratio. Century RNA markers (Amion) were used as molecular size standards.

**RESULTS AND DISCUSSION**

Identification of Two Alternatively Spliced loop II–III Variants of Cav1.2 Ca\(^{2+}\) Channels—The second intracellular loop connecting domains II and III of the \(\alpha_1\) subunit was generously provided by Dr. Kathleen Sweadner. Membrane fractions from rat myocardium were isolated by differential and sucrose density gradient centrifugation and characterized for the enrichment of markers for the sarcolemma (~20-fold enrichment in Na\(^{+}/K\)-ATPase and adenylate cyclase activities) and heavy sarcoplasmic reticulum (~10-fold enrichment in ryanodine receptors) as described (67). Membrane proteins were subjected to SDS-polyacrylamide gel electrophoresis followed by transfer to nitrocellulose membranes, which were probed with affinity-purified antipeptide antibodies.

Screening of Recombinant Clones and Cycle Sequencing—Recombinant clones of loop II–III RT-PCR amplicons subcloned into either pCR1 or pTZ18 were selected and inouculated overnight at 37 °C in LB broth containing 75 \(\mu\)g/ml ampicillin. Plasmid DNA was isolated using an alkaline miniprep (49). The recombinant constructs were screened by restriction enzyme digestion and gel electrophoresis to identify loop II–III deletion variants. The various inserts were then purified with a Qiagen miniprep plasmid preparation method to obtain high-purity templates suitable for cycle sequencing with the Applied Biosystems Prism dye terminator cycle-sequencing method (PerkinElmer Life Sciences), and the sequences were analyzed using SeqAidII tools (University of Utah).
represents a PCR-negative control. Subcloning and direct sequencing of the PCR products shown in lane 1 revealed that the 452-nt product matched perfectly the sequence of the intracellular loop linking domains II and III of the rabbit heart α1 Cav1.2 subunit gene, whereas that of the smaller diffuse fragment matched the 452-nt loop II–III sequence except for a deletion of 133 nt (from 2812 to 2945). Open reading frame analysis of the 320-nt amplicon containing the 133-nt deletion indicated that the deletion was predicted to shift the translational reading frame of full-length α1 Cav1.2 and to introduce a premature chain termination at nt 2995 (data not shown). To obtain longer cDNAs encompassing the entire loop II–III and the predicted stop codon, RT-PCR was performed with another...
set of primers nested in the transmembrane segment S6 of domain II and S3 segment of domain III (loop 5', nt 2509–2529; and loop 3', nt 3199–3179, respectively). Primers were designed to contain BamHI and EcoRI restriction enzyme sites to facilitate subcloning and analysis of the PCR amplicons. Fig. 1B depicts RT-PCR of rabbit heart, where the first-strand cDNA was performed with the gene-specific antisense oligonucleotide loop 3', and PCR amplification was carried out with loop 5' and loop 3' primers. Similar to RT-PCR analysis carried out with the JW5 and JW6 primers, amplification with the loop 5' and loop 3' primers produced two products, an amplicon of ~700 nt and a lower band of ~560 bp (Fig. 1B, lane 1). Moreover, the size difference between the top and bottom products was ~140 nt, analogous to the RT-PCR performed with the JW5 and JW6 primers. After subcloning of the amplified products, three representative amplicons were characterized by restriction mapping and direct sequencing (Fig. 1C). Lane 1 shows the presence of a 690-nt product, representing the expected amplification product of α1 Ca1.2 with the loop 5' and loop 3' primers, and lanes 2 and 3 represent two clones isolated and characterized from the lower RT-PCR amplification band. The nucleotide sequence analysis and alignment shown in Fig. 2A indicated that the 690-nt product represented the nucleotide sequence of the expected amplified fragment encompassing the wild-type intracellular loop II–III of Ca1.2 (Rab-H), whereas the smaller products denoted two distinct variants of this sequence. Variant 1, denoted RH-1, and variant 2, denoted RH-2, appear to be derived from the full-length loop II–III sequence spanned by the loop 5' and loop 3' primers, as a consequence of internal deletions of 133 and 130 nt, respectively. The RH-1 variant contained deletion of nt 2812–2945, and it represents a deletion variant already characterized in the RT-PCR analysis carried out with the JW5 and JW6 primers. The RH-2 variant displayed deletions of nt 2681–2811, and it represents a novel variant cloned from the smaller amplification product of loop II–III. Analysis of the open reading frame of the deletion variants RH-1 and RH-2 in relation to the wild-type sequence confirmed that both deletions shifted the open reading frame, thus introducing a common termination codon (TGA) at nucleotide 2995. The amino acid sequence of the variant proteins was as follows: RH-1 contained a deletion of amino acids 933–1066, and RH-2 contained a deletion of amino acids 933–1063.
alignment of the variants indicated that both variants introduce novel amino acid sequences past their respective deletions depicting novel carboxyl terminal sequences of the truncated polypeptides (Fig. 2B). Because both variants use the same stop codon, the terminal 19 amino acids were identical in the two variants. An independent isolation of the RH-1 and RH-2 cDNAs was obtained with RT-PCR of RNA from the whole heart and cardiac left ventricle but not skeletal muscle.

**Evaluation of Genomic Organization of the Loop II–III Variants**—The comparison of the deletion sites of the loop II–III variants with the exon-exon boundaries of the human α1,2 subunit gene is summarized (Fig. 2C). Depicted are the exons encoding loop II–III of the α1,2 subunit gene as determined by Soldatov (57) and how the variants would potentially arise as a result of internal deletions. The RH-1 variant lacks exon 19, which results in a reading frameshift of exon 20, thus generating a novel 19-amino acid carboxyl-terminal peptide with an in-frame premature termination codon. The RH-2 variant, on the other hand, could arise because of the deletion of a 3′ 60-nt fragment of exon 17 and the entire exon 18. The putative alternative 5′ donor site of the variant would be in exon 17, whereas the 3′ splice site of exon 19 would be used as a 3′ acceptor. The deletion of the 3′ part of exon 17 and the entire exon 18 predicts a reading frameshift, such that exons 19 and 20 are translated with respect to a new reading frame. Consequently, exons 19 and 20 are predicted to translate a novel 62-amino acid carboxyl-terminal polypeptide.

**mRNA Expression of Cav1,2 Ca2+ Channel Variants**—Confirmation of the expression of the Ca1,2 subunit mRNA variants detected by RT-PCR was sought by RNase protection assays. Fig. 3 represents a schematic of the predicted sizes of the protected fragments that would be expected with the RH-1 and RH-2 probes. An antisense RNA probe of RH-1 was constructed based on the 323-nt amplicon of loop II–III (exons 16–18 and 20). The mRNA from cardiac and fast twitch skeletal muscle was used to protect the RH-1 probe, and a representative autoradiogram is shown. The mRNA from cardiac muscle protected the 323-nt probe representing the RH-1 variant as well as fragments of ~274, 144, and 46 nt (Fig. 3, lane 3). The 323-nt protected fragment represents the RH-1 type variant of loop II–III and would correspond to a part of exon 16 through to the end of exon 18 and the beginning of exon 20. Exon 19 of 133 nt is deleted in the RH-1 splice variant. The 274-nt protected fragment denotes a part the of the wild-type α1 subunit loop II–III, from exons 16 to 18. Because exon 19 is spliced out in the RH-1 type variant, this exon is unspliced and consequently degraded by RNase. The theoretically expected fragments of 144 and 46 nt, which would be contributed by the RH-2 variant and the 3′ end of the wild-type loop II–III encoded by part of exons 16 and 17 and the entire exon 18 are deleted in the RH-2 type variant, this region of loop II–III would not be protected and consequently degraded by RNase. The theoretically expected fragments of 144 and 46 nt contributed by the 3′ end of the wild type loop II–III (exon 16 and the 5′ end of exon 17) and the RH-1 deletion variant were also detected but at low levels.

**Brain mRNA also protected multiple fragments by RNA from cardiac muscle is suggestive of the expression of the RH-1and RH-2 deletion variant transcripts as well as the wild type loop II–III. To estimate a relative level of expression of the three transcripts, the ratio of RH-1 and RH-2 deletion variants to the wild-type Cav1.2 loop II–III was quantified by using the relative band intensities of the RH-1 (320-bp band) and the RH-2 (323-bp band) protected fragments and the appropriate protected fragments contributed by the wild type loop II–III from the autoradiogram, 274 and 174 bp, respectively. After normalization, the analysis estimated a relative level of expression with a ratio 1:1.6:0.7 of wild-type loop II–III:RH-1:RH-2, respectively; however, the estimate remains to be confirmed with protein expression levels.

**Brain mRNA also protected multiple fragments by RNA from cardiac muscle is suggestive of the expression of the RH-1and RH-2 deletion variant transcripts as well as the wild type loop II–III. To estimate a relative level of expression of the three transcripts, the ratio of RH-1 and RH-2 deletion variants to the wild-type Cav1.2 loop II–III was quantified by using the relative band intensities of the RH-1 (320-bp band) and the RH-2 (323-bp band) protected fragments and the appropriate protected fragments contributed by the wild type loop II–III from the autoradiogram, 274 and 174 bp, respectively. After normalization, the analysis estimated a relative level of expression with a ratio 1:1.6:0.7 of wild-type loop II–III:RH-1:RH-2, respectively; however, the estimate remains to be confirmed with protein expression levels.**
RNase protection assays are indicative of the expression of these variants in cardiac and neuronal tissue but not skeletal muscle and are therefore consistent with the reported pattern of expression of the \( \text{Ca}_{1.2} \) \( \alpha_1 \) subunit gene.

**Domains I and II as Potential Structural-Functional Units of \( \text{Ca}^{2+} \) Channels**—On the basis of the identification of the loop II–III deletion variants, it was hypothesized that the alternatively spliced forms of \( \alpha_1 \) subunit of RH-1 and RH-2 would generate two-domain truncated forms of the \( \text{Ca}_{1.2} \) \( \alpha_1 \) subunit. To verify the hypothesis, the full-length cDNAs encoding two-domain polypeptides needed to be selectively isolated. RT-PCR of cardiac RNA was performed with a common 5' sense primer (nt 180–203, TGGAAACTGACAATGCTTCGAGCC) and the respective splice junction primers selective for the RH-1 and RH-2 variants (ATCCTCTTCTCCTGGCTC-CTC-3' and 5'-GAGGCAGGAACCTGTTGTTTCC, respectively). RT-PCR yielded two products of \( 2.6 \) (Fig. 4, lane 1) and \( 2.4 \) (Fig. 4, lane 2) kb, representing RH-1 and RH-2 variants, respectively, which were subsequently TA cloned and then cycle sequenced. Lane 3 represents a PCR-negative (water) control. A control with RNA but without RT (for contamination with genomic and partially processed mRNA) was also run, and it was negative.

The nucleotide sequence and the deduced amino acid sequence analysis of the RH-1 and RH-2 variants are shown in Fig. 5, which indicates identical nucleotide and amino acid sequences in domains I and II when aligned with the wild-type \( \text{Ca}_{1.2} \) \( \alpha_1 \) subunit. The salient sequence differences were present in loop II–III as indicated. The RH-1 variant would generate a polypeptide of predicted molecular mass of 99.0 kDa, whereas the RH-2 variant would encode a polypeptide of 99.2 kDa.

Confirmation of the \textit{in vivo} expression of the two-domain \( \text{Ca}_{1.2} \) \( \alpha_1 \) subunit gene was carried out by Western blot analysis of subcellular fractions from cardiac muscle with antipeptide antibodies specific for loop II–III (CNC-1) of the \( \text{Ca}_{1.2} \) calcium channel. The peptide sequence used as antigen is also preserved in the RH-1 and RH-2 variants. Subcellular fractions enriched in SL and HSR were immunoblotted with anti-Na\(^{+}/K\(^{+}\)-ATPase monoclonal antibody to confirm the enrichment of SL versus the SR (Fig. 6). The Na\(^{+}/K\(^{+}\)-ATPase staining is highly enriched in SL compared with the original microsomes and fraction HSR, and densitometry revealed that it was \( 15\)-fold higher in SL compared with the other fractions. Immunoblotting with the affinity-purified CNC-1 antibody indicated the presence of an \( 240\)-kDa polypeptide representing the \( \alpha_1 \) Cav1.2 subunit in microsomes (Fig. 6, lane R) that was found to enrich in SL, although a substantial amount (\( \sim 40\% \)) of this immunoreactive polypeptide was also present in HSR. Interestingly, the CNC-1 antibody also recognized an \( 99\)-kDa polypeptide in the microsomal fractions that was found to enrich in HSR but was completely absent from the sarcolemmal fraction. The immunoreactivity of CNC-1 with the
Alternative Splicing of the \( \alpha_1 \) Subunit of \( \text{Ca}_{\text{v}}1.2 \)

The predicted topology of the two-domain \( \alpha_1 \), \( \text{Ca}_{\text{v}}1.2 \) subunit variants generated by alternative splicing from the proposed four-domain \( \text{Ca}^{2+} \) channel structure is shown (Fig. 7). The full-length RH-1 variant renders a truncated, two-domain polypeptide composed of normal domains I and II, as well as 78 amino acids of the wild-type loop and a novel 19-amino acid carboxyl-terminal tail. The RH-2 variant, on the other hand, contains normal domains I and II and 35 amino acids of the wild-type loop II–III and a unique 62-amino acid carboxyl-terminal peptide. The terminal 19 amino acids in both variants would be identical.

The intracellular loop of \( \alpha_1 \) subunits connecting domains II and III of the \( \text{Ca}^{2+} \) channel family of proteins is a critical determinant of the mode of signal transduction through protein-protein interactions (45–47, 50–57), which can be further modulated by post-translational mechanisms such as phosphorylation of amino acid residues in this loop (51, 58–60). Although the consensus sequence prediction program PROSITE predicts putative PKC (TTK) and casin kinase 2 (TTGE) phosphorylation sites in the wild-type loop II–III, the demonstration of their phosphorylation and any functional consequences remains to be determined. Interestingly, however, the putative PKC site is deleted in the RH-2 variant, and the casin kinase 2 site is deleted on both RH-1 and RH-2, whereas the novel carboxyl termini introduce additional consensus PKC sites, one in RH-1 and three in RH-2. In view of the significant changes in the amino acid composition and perhaps tertiary structure of loop II–III in the two variants, it is speculated that these polypeptides (if functional) could markedly influence signal transduction via these structural units.

Although the role of the \( \alpha_1 \) subunit variants producing truncated, two-domain \( \text{Ca}^{2+} \) channel polypeptides remains to be elucidated, existence of other two-domain channel polypeptides in the voltage-dependent superfamily has also been reported. A two-domain, truncated form of \( \text{Ca}_{\text{v}}1.1 \alpha_1 \) subunits comprising domain I and chimeric domain IV generated by splicing of the S2 segment of domain II to the S2 segment of domain IV has been observed predominantly in muscle from neonates (61). In this regard, we observed that the RH-1 and RH-2 variants were expressed in fetal and neonatal hearts (data not shown). Genetic mutations of the neuronal \( \text{Ca}_{\text{v}}2.1 \) (P/Q-type \( \text{Ca}^{2+} \) channel, \( \text{CACNL1A4} \)), associated with inherited episodic ataxia, predict synthesis of a truncated, two-domain channel polypeptide composed of domains I and II and a part of domain III, suggesting that these molecules have functional consequences (62–64). In addition, a voltage-dependent \( \text{Na}^{+} \) channel \( \alpha \) subunit, \( \text{SCN8A} \), generates a predicted polypeptide comprising domains I and II as a result of alternative splicing of exon 18N, which introduces an in-frame termination codon (65). The predicted topography of truncated \( \text{CACNL1A4} \) is similar to that proposed for the \( \text{SCN8A} \) \( \text{Na}^{+} \) channel polypeptide. Recent studies revealed that the \( \omega \)-conotoxin MVIIIC receptor associated with \( \text{Ca}_{\text{v}}2.1 \) (P/Q-type) \( \text{Ca}^{2+} \) channels consisted of a 95-kDa polypeptide that comprised domains I and II and a part of loop II–III (66). The 95-kDa polypeptide was able to bind the auxiliary \( \beta \) subunits, and it remains to be determined whether the polypeptide can form functional channels. Furthermore, it would be intriguing to elucidate whether the 95-kDa polypeptide is also generated by the alternative splicing mechanisms of the \( \text{Ca}_{\text{v}}2.1 \alpha_1 \) subunit gene, similar to the \( \text{Ca}_{\text{v}}1.2 \) gene. It is also notable that a unique cDNA has been isolated from kidney that encodes a two-domain polypeptide that exhibits 20% identity with the voltage gated \( \text{Na}^{+} \) and \( \text{Ca}^{2+} \) channels (69).

The generation of two distinct polypeptides by alternative splicing is highly suggestive of functional specialization of the heavy SR membrane in cardiac muscle but not the SL.
individual truncated \( \alpha_1 \) subunit isoforms described here. The presence of the critical determinants of channel function, including activation and inactivation domains, the voltage sensor, ion selectivity, and pore structure within domains I and II, suggests that the truncated channel polypeptides could perhaps homodimerize and heterodimerize to form functional channels. The RH-1 and RH-2 variants may also play roles as dominant negative regulators of the wild type Cav1.2 \( \mathrm{Ca}^{2+} \) channels. It is notable that subcellular distribution of the 240-kDa \( \alpha_1 \) subunit indicated the presence of a substantial pool of this isoform in the heavy SR membranes, probably representing the dyad structure known to be enriched in this fraction. The exclusive presence of the 99-kDa RH-1 and RH-2 variants in this fraction suggests that these polypeptides may play a role in excitation–contraction coupling at the dyad junctions. In this regard, purified dyads were found to be highly enriched in both the 240- and 99-kDa polypeptides (data not shown). The RH-1 and RH-2 polypeptides may also serve regulatory roles by sequestering auxiliary channel subunits, such as the \( \beta \) subunit, because the binding site for this modulator would be retained by the two variants. In view of heterogeneity of the tissues in which the Ca,1.2 \( \alpha_1 \) subunits are expressed, it is conceivable that the splice variants may also be present in a subset of cells geared for specialized regulation of calcium entry. The level of expression of the two variants compared with the wild type is indicative of a stoichiometric relationship between the various isoforms. Studies indicate that there may be a voltage-dependent component to the excitation–contraction coupling mechanism in cardiac tissue (70), and whether the two domain polypeptides serve a role in this process remains to be defined.

In summary, the definition of the two new splice variants of the \( \alpha_1 \) subunit gene, which form two-domain polypeptides, implies a novel mechanism for \( \mathrm{Ca}^{2+} \) channel structure and function or regulation under normal physiological conditions. Moreover, these results lend further support for an evolutionary model of a four-domain channel structure created by two subsequent gene duplications of an ancestral one-domain gene.

REFERENCES

10. Sobko, A., Puretz, A., Shirshai, O., Mijkin, A., Cherepanova, V., Dagan, D., and

Fig. 7. Topology of full-length \( \alpha_1 \) Cav1.2 and the deletion variants (RH-1 and RH-2). The topology of the full-length cardiac Cav1.2 \( \mathrm{Ca}^{2+} \) channel and the predicted topology of the two-domain channels are shown. The RH-1 two-domain channel arises as a result of alternative splicing of exon 19, which results in reading frame shift of exon 20 and a premature termination codon. The RH-2-type channel, on the other hand, is generated by deletion of a fragment of exon 17 and entire exon 18, which results in a reading frameshift of exons 19 and 20. RH-1 and RH-2 both share a common 19-amino acid carboxyl terminus; however, RH-2 in addition contains a unique 43-amino acid sequence. The two-domain polypeptides retain the critical determinants of channel function, including activation, inactivation, voltage sensor, ion selectivity, and pore structure, which are contained within domains I and II. The \( \beta \) subunit binding sites will also be retained in the two variants, whereas the ligand binding sites for phenylalkylamines (PAA), the benzothiazepines (BTZ), and the dihydropyridines (DHP) will not be retained. The putative carboxyl-terminal PKC phosphorylation sites are indicated.
Alternative Splicing of the α1 Subunit of Ca\textsubscript{v,1.2}
Alternative Splicing in Intracellular Loop Connecting Domains II and III of the α1 Subunit of CaV1.2 Ca2+ Channels Predicts Two-domain Polypeptides with Unique C-terminal Tails

Paul A. Wielowieyski, Jeffrey T. Wigle, Maysoon Salih, Peggy Hum and Balwant S. Tuana

doi: 10.1074/jbc.M006868200 originally published online September 28, 2000

Access the most updated version of this article at doi: 10.1074/jbc.M006868200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 69 references, 25 of which can be accessed free at http://www.jbc.org/content/276/2/1398.full.html#ref-list-1