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Calcium Channel $Ca_V\alpha_1$ Splice Isoforms - Tissue Specificity and Drug Action

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Abstract

Voltage-gated calcium ion channels are essential for numerous biological functions of excitable cells and there is wide spread appreciation of their importance as drug targets in the treatment of many disorders including those of cardiovascular and nervous systems. Each *Cacna1* gene has the potential to generate a number of structurally, functionally, and in some cases pharmacologically unique $Ca_V\alpha1$ subunits through alternative pre-mRNA splicing and the use of alternate promoters. Analyses of rapidly emerging deep sequencing data for a range of human tissue transcriptomes contain information to quantify tissue-specific and alternative exon usage patterns for *Cacna1* genes. Cell-specific actions of nuclear DNA and RNA binding proteins control the use of alternate promoters and the selection of alternate exons during pre-mRNA splicing, and they determine the spectrum of protein isoforms expressed within different types of cells. Amino acid compositions within discrete protein domains can differ substantially among Ca_V isoforms expressed in different tissues, and such differences may be greater than those that exist across Ca_V channel homologs of closely related species. Here we highlight examples of Ca_V isoforms that have unique expression patterns and that exhibit different pharmacological sensitivities. Knowledge of expression patterns of Ca_V isoforms in different human tissues, cell populations, ages, and disease states should inform strategies aimed at developing the next generation of Ca_V channel inhibitors and agonists with improved tissue-specificity.

Keywords

Alternative splicing; *Cacna1* genes; dihydropyridines; morphine; splicing factors; voltage-gated calcium channels; ziconotide

1. INTRODUCTION

The functional core of the voltage-gated calcium ion channel is the $Ca_V\alpha_1$ subunit and each may be associated with a cytoplasmic calcium channel $Ca_V\beta$ subunit, an extracellular $Ca_V\alpha_2\delta$ subunit, and other proteins such as gamma subunits, calmodulin, scaffold proteins, kinase anchoring proteins, and downstream target proteins [1]. In mammals, 10 different genes (*Cacna1A-1I* and *1S*) encode 10 $Ca_V\alpha_1$ subunits, and in humans these reside on nine

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CONFLICT OF INTEREST

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different chromosomes. *Cacna1* genes encode three main classes of $Ca_V\alpha_1$ proteins grouped according to their degree of sequence homology: Ca_V1 (*1S, 1F, 1C, 1D*), Ca_V2 (*1A, 1B, 1E*) and Ca_V3 (*1G, 1H, 1I*) [2–4]. Expansion within each of the 3 main *Cacna1* gene families likely occurred some 450 million years ago probably as a result of genome doublings from single ancestral vertebrate *Cacna1* genes [5]. For example, phylogenic analyses suggest that *Cacna1s*, *Cacna1c*, *Cacna1d*, and *Cacna1f* genes are located within paralogous chromosome regions (spread across four different chromosomes) that contain several gene families including visual opsins [5].

As individual calcium channel genes evolved, Ca_V proteins acquired new functions and phenotypes that account for their unique biological activities in specific tissues and cell types. For example, of the four mammalian *Cacna1* genes that encode Ca_V1 subunits: *Cacna1s* expression is limited to skeletal muscle and $Ca_V1.1$ proteins contain a unique sequence in the II–III linker region that couples the channel to the ryanodine receptor. This is the critical first step in excitation-contraction coupling in skeletal muscle [6–8]; *Cacna1f* is highly expressed in retina but also in other human tissues (*CACNA1F*, the GTEx Portal on 12/06/2014) [9], and $Ca_V1.4$ channels are important for transmission at glutamatergic ribbon synapses between photoreceptors and second-order neurons [10–13]; and *Cacna1c* and *Cacna1d* genes have relatively broad expression patterns including in brain, heart, smooth muscle, endocrine cells, and auditory hair cells [1, 14] (*CACNA1C* and *CACNA1D*, the GTEx Portal on 12/06/2014) [9]. The expression of functionally different splice isoforms of $Ca_V1.2$ and $Ca_V1.3$ in different tissues enables *Cacna1c* and *Cacna1d* genes to support a wider range of calcium-dependent cellular functions [2, 3, 15–17].

2. PHARMACOLOGICAL TOOLS THAT DISCRIMINATE AMONG MAJOR SUBTYPES OF Ca_V CHANNELS

Pharmacological approaches are invaluable to delineate the cellular functions of different *Cacna1* genes. For example, dihydropyridine (DHP) antagonists and agonists are the molecules of choice to establish the contributions that Ca_V1 channels make to various cellular responses, whereas the spider toxin ω-agatoxin IVA and the cone snail toxin ω-conotoxin GVIA are highly selective inhibitors of $Ca_V2.1$ and $Ca_V2.2$ channels respectively (Table 1). As is well known, and discussed throughout this special issue, DHP antagonists are used in the clinic to lower blood pressure. All four Ca_V1 channels are sensitive to DHPs, but their anti-hypertensive action *in vivo* is dominated by the inhibitory actions of several DHPs on vascular smooth muscle, DHPs have few side effects, a surprising fact considering the relatively broad expression patterns of $Ca_V1.2$ and $Ca_V1.3$ channels in many excitable cells.

As shown by Bruce Bean, and by Reuter and colleagues, the efficacy of DHP antagonists is strongly voltage-dependent and these drugs are more potent inhibitors of Ca_V1 channels that are activated from relatively depolarized membrane potentials typical of smooth muscle cells *in vivo* [18, 19]. Preferential inhibition of inactivated Ca_V1 channels also results in reduced inhibition by DHPs of Ca_V1 channels activated by brief, non-inactivating stimuli applied from negative membrane potentials – such as action potential-like waveforms [20]. $Ca_V1.3$

channels have different biophysical properties and have somewhat lower intrinsic sensitivities to certain DHPs as compared to $Ca_V1.2$ channels [21, 22]; although these differences are generally insufficient to permit unambiguous functional separation of $Ca_V1.2$ and $Ca_V1.3$ currents at cellular and behavioral levels. $Ca_V1.3$ channels support low threshold calcium entry during pacemaking in some cells [23–25] and data link *de novo* mutations in *CACNA1D* to increased risk of sporadic autism spectrum disorder [26]. Risk alleles for bipolar disorder, schizophrenia and other psychiatric illnesses have also been linked to *CACNA1C* [27–30]. Such findings have increased efforts to develop compounds to discriminate between $Ca_V1.2$ and $Ca_V1.3$ channels, as well as among their various splice isoforms. There is also evidence discussed below, that splice isoforms of $Ca_V1.2$ have different sensitivities to DHPs.

3. CELL-SPECIFIC ALTERNATIVE PRE-mRNA PROCESSING

All mammalian *Cacna1* genes studied to date contain alternatively spliced exons and many have alternative promoters; these exons combine in different patterns according to cell-specific control, with the potential to generate thousands of Ca_V channels [2]. We know that alternative splicing of *Cacna1* pre-mRNAs is extensive in mammals, but we still have only a cursory understanding of their cell-specific functions. Recent reviews have summarized what was known of the tissue-specific expression pattern of exon usage for several *Cacna1* genes and, where tested, their functional impact on Ca_V channel properties [2, 15, 31, 32]. More recently, the Genotype-Tissue Expression (GTEx) project provides the most comprehensive publicly available dataset on the expression pattern of human genes, including *CACNA1*, and the quantitative comparison of individual exon usage, genome-wide, across different tissues [9].

Multi-exon genes have the capacity to generate several – sometimes thousands – of proteins with unique structures through cell-specific selection (or elimination) of certain exons. Alternatively spliced exons often encode autonomous protein modules that introduce or eliminate specific functions or that promote or inhibit coupling to downstream signaling cascades [33–35]. Cell-specific exon choice can involve the inclusion or exclusion of a cassette exon, the choice of one of a pair or more of mutually exclusive exons, or the use of alternate splice donor or acceptor sites (Fig. 1). Cell-specific inclusion or exclusion of certain exons can also introduce premature termination codons that promote nonsense-mediated decay (NMD) thereby regulating the overall protein expression level (Fig. 1). For example, e20a in *Cacna1b* introduces a premature stop codon if inserted during pre-mRNA splicing and $Ca_V2.2$ mRNAs containing this exon are present in brain of mice and human (Fig. 1) (GenBank *FJ609386.1*; see also *CACNA1B* in GTEx portal [9]). Alternative splicing-linked NMD is common during early differentiation of cell-types and often involves the family of polypyrimidne tract binding protein (PTB) splicing factors, and NMD has recently been shown to involve the action of neuronal splicing factors of the neuro-oncological ventral antigen (NOVA) family, that regulate splicing of cryptic exons within introns [33, 36–38].

Recent large-scale transcriptome sequencing projects suggest that alternative exon usage (pre-mRNA splicing and alternate promoters) occurs in >97% of multi-exon genes and it is

extensive in mammalian brains [39–43]. Cell-specific splicing (inclusion or exclusion) of alternative exons in the nervous system is essential for development, axon targeting, neuronal excitability, circuit formation, and drug action [2, 3, 42–46]. Cell-specific splicing factors determine when and which Ca_V channel isoforms are expressed in a given cell type [38, 41, 47–49]. Furthermore, upstream factors regulate the expression and activity of cell-specific splicing factors including other splicing factors, miRNAs, kinases, as well as epigenetic modifiers [38, 50–52].

Cell-specific splicing factors work in concert with the ubiquitous splicing machinery to select (or skip) optional exons during pre-mRNA processing (Fig. 2) (Table 1) [33, 38, 40, 53, 54]. The expression patterns and activities of cell-specific splicing factors differ according to cell-type, age, activity, and potentially metabolic state and disease states [33]. Certain master splicing factors control the selection of exons that are essential for cell-fate determination, whereas others regulate exon choice that exert control over specific cell-functions [38]. Tremendous progress has been made in mapping splicing factor binding on a genome-wide scale, leading to the identification of splicing networks for NOVA, PTB, and rbFOX and inevitable insights into genome-wide biological significance [38, 40, 55, 56]. Splicing factors known to regulate cell-specific splicing of Ca_V pre-mRNAs are summarized in Table 1.

Here we focus on a few examples from three *Cacna1* genes to illustrate approaches that are being employed to assess the pharmacological consequences of alternative splicing on Ca_V channels, in expression systems and within their native environment *in vivo*. Other reviews illustrate the capacity of alternative splicing to stratify Ca_V channel phenotypes modifying biophysical characteristics, membrane trafficking, subcellular targeting, association with downstream target proteins, and pharmacology [2, 3, 15].

4. SPLICE ISOFORMS EXPRESSED IN DIFFERENT TISSUES ARE DIFFERENT

Most, although not all, alternatively spliced cassette exons in *Cacna1* genes are located in hyper-variable domains of Ca_V proteins that can accommodate as few as one amino acid or larger insertions comprised of several amino acids, without disrupting basic protein function. Cassette exons are either inserted or skipped and they are located in the intracellular C-termini and N-termini, intracellular I–II, II–III, and III–IV linkers, as well as S3-S4 extracellular linkers in domains III and IV of Ca_V channels [2, 3, 15]. Pairs of mutually exclusive exons often encode regions that do not tolerate large change in protein structure, and these have been shown to modify amino acid composition in transmembrane spanning domains including IS6, IIIS2, IVS3 and the proximal region of the C-terminus of Ca_V channels (Fig. 3) [2, 3, 15].

Certain alternatively spliced exons are conserved among *Cacna1* genes. For example, all but one *Cacna1* gene contains an alternatively spliced cassette exon that encodes a region in the putative extracellular linker between transmembrane spanning helices S3 and S4 in domain IV of Ca_V channels (e.g. e29 in *Cacna1s*; e31a in *Cacna1a*; e31a in *Cacna1b* and e33 in *Cacna1c* [3] and GTEx portal [9]). The composition and length of these exons differ across

genes, but all affect voltage-dependent gating of Ca_V channels [3]. Many other alternatively spliced exons influence channel gating including the voltage dependence of channel activation and inactivation, gating kinetics, and calcium-dependent inactivation [57–67].

Analyses of tissue-specific transcriptomes across mammals in a variety of tissues have demonstrated that alternative splicing is also frequently lineage-specific such that there is variation in the splicing of alternative exons between species [55]. This finding emphasizes the importance of study the pattern of alternative splicing in human tissue, if the ultimately goal is drug development for human use. Interestingly, transcriptome-wide analyses across several species of mammals also show that alternative splicing frequently alters the degree of protein phosphorylation implicating an important role in defining second messenger signaling cascades [55]. It is also important to point out, however, that difference in amino acid composition and protein function among Ca_V splice isoforms, generated from the same gene but expressed in different tissues, can also be substantial in highly variable domains including C-termini. Indeed, tissue-specific differences in amino acid compositions among Ca_V channel isoforms generated from one gene may be greater than those that exist across Ca_V channels of the same family across closely related species. This is especially dramatic for large alternative exons. For example, several *Cacna1* genes have different tissue-specific promoters that generate $Ca_{V1}\alpha$ subunits with substantially different N-termini. Exon 1a of *Cacna1c* is expressed in heart and encodes 46 amino acids defining the start of the N-terminus, whereas in other tissue including brain and smooth muscle, exon 1b is expressed and it encodes a completely different 16 amino acid sequence [68–70]. By contrast, exons 1a and 1b are highly conserved across mammals and vertebrates (see multi-alignment of vertebrates in UCSC genome browser [71] and GTEx portal [9]). Similarly, exon 18a of *Cacna1b*, and an equivalent exon in *Cacna1e*, encode 21 amino acids in the II–III linkers of $Ca_V2.2$ and $Ca_V2.3$ respectively. The amino acid sequences of these alternative exons and of constitutive flanking exons, are highly conserved across species, but expression patterns of the alternative exons differ according to tissue and development [45, 71].

5. SPLICE ISOFORMS ARE TISSUE-SPECIFIC AND CELL-SPECIFIC

By definition, cell-specific processing of pre-mRNAs results in the expression of unique patterns of Ca_V isoforms in different cells and different tissues. This offers the opportunity to achieve a higher level of tissue targeting that might be achieved otherwise, if drugs can be identified that differentially act on splice isoforms. Indeed, the therapeutic efficacy of certain drugs can depend on their preferential action on specific splice isoforms, such as the action of non steroidal anti-inflammatory drugs that have different therapeutic profiles that depend on their differential action on isoforms of cyclooxygenase (COX-1, COX-2, and COX-3) [72–75].

Within Ca_V1 channels, there is interest in identifying drugs that target brain-specific $Ca_V1.2$ channel isoforms over those that dominate in cardiac and smooth muscle. The precise composition and relative abundance of $Ca_V1.2$ isoforms across different human tissue can now be analyzed from transcriptome sequencing projects (GTEx portal [9]). Certain *CACNA1C* exons appear to be preferentially expressed in heart including e1a (which is

controlled by a cardiac tissue-specific promoter; [68, 76]), while other exons might be preferentially expressed in brain or smooth muscle [15].

6. ALTERNATIVE SPLICING OF Ca_V PRE-mRNAs INFLUENCE DRUG ACTION

6.1. Splice Isoforms of $Ca_V1.2$ have Different Sensitivities to DHPs

In 1995 Soldatov, Bouron, and Reuter published an important paper showing - for the first time - that different splice isoforms of $Ca_V1.2$ channels exhibit measurable differences in their biophysical as well as pharmacological properties. They suggested that the different actions of DHP antagonists on splice isoforms of $Ca_V1.2$ might account for some of their higher selectively on smooth muscle channels [77, 78] (Fig. 3). Three pairs of mutually exclusive exons in *Cacna1c* have been reported to modify the sensitivity of $Ca_V1.2$ calcium currents to DHPs: e8a/e8, e21/e22 and e31/e32 (>10-fold changes). While, more subtle effects of DHPs and other calcium channel blockers on inhibition of Ca_V1 channels (less than 10-fold changes in potency), have been reported for e9* of *Cacna1c* (diltiazem) [79] and for e42a of *Cacna1d* (nimodipine) [80].

E8a and e8 encode sequences of IS6 and part of the S5–S6 pore forming loop; photoaffinity studies indicate that the extracellular portion of this region can interact with DHPs [81–83]. Inclusion of e8 over e8a increases the sensitivity of $Ca_V1.2$ currents to DHPs [81, 84]. Interestingly, e8 and e8a have different patterns of tissue expression in mammals. E8 expression is higher in lungs and aorta than in cardiac muscle, while e8a usage is more prevalent in heart than smooth muscle of rodents and human [9, 81, 85]. This observation may also contribute to the dominant biological actions of certain DHPs as anti-hypertensive [86].

Cacna1c e21 and e22 are mutually excusive and each encodes part of the extracellular linker IIIS1–IIIS2 as well as most of the IIIS2 transmembrane helix of $Ca_V1.2$. As discussed above, e22 increases the sensitivity of $Ca_V1.2$ channels to DHP antagonists compared to e21 [77, 78, 85]. E21 and e22 are expressed throughout heart, aortic tissue and brain [9, 85, 87], although e22 is relatively more abundant in rat cardiac ventricular muscle than in aortic tissue [85]. Mutually exclusive *Cacna1c* exons e31 and e32 each encode most of the intracellular IVS2–IVS3 linker and the entire IVS3 transmembrane helix of $Ca_V1.2$. In heart, the selection of e31 over e32 (and *vice versa*) varies with development and disease state [88]. E31 dominates early in development and it reemerges in heart failure [89, 90], while e32-containing $Ca_V1.2$ mRNAs dominate in normal adult heart [89].

6.2. Splice Isoforms of $Ca_V2.1$ have Different Sensitivities to ω-Agatoxin IVA

Zamponi and colleagues showed that the actions of ω-agatoxin IVA are influenced by the presence of a two amino acid sequence, NP, encoded by e31a of the *Cacna1a* gene. This sequence comprises part of the putative extracellular linker between domains IVS3 and IVS4 of $Ca_V2.1$ [61]. $Ca_V2.1$ channels including NP are up to 11-fold less sensitive to ω-agatoxin IVA inhibition compared to $Ca_V2.1$ channels that lack NP. $Ca_V2.1$ mRNAs containing e31a are more abundant in Purkinje cells than in cerebellar granule cells; a result

that nicely parallels the greater sensitivity of so called P-type currents, as compared to Q-type currents, to ω-agatoxin IVA [61]. The brain-specific splicing factor NOVA-2 represses the inclusion of e31a during alternative splicing of $Ca_V2.1$ pre-mRNA, and notably has the opposite action on the homologous e31a of *Cacna1b* [49]. NOVA-2 is known to be essential for coordinating cell-specific alternative splicing among a network of genes critical for synaptic function [91, 92].

6.3. Splice Isoforms of $Ca_V2.2$ have Different Sensitivities to G Protein Coupled Receptors

$Ca_V2.2$ channels underlie N-type currents that carry calcium entry into presynaptic terminals to control transmitter release from many synapses including primary nociceptive afferent synapses to dorsal horn neurons in spinal cord [93–98]. $Ca_V2.2$ channels are important drug targets that act directly to inhibit channel activity, as well as indirectly *via* G protein coupled receptors (GPCRs). Ziconotide (also known as ω-conotoxin MVIIC) is analgesic in humans and rodents when administered by intrathecal injection and it can prevent, as well as reverse symptoms of chronic pain induced by inflammation or nerve injury [99, 100]. In spinal cord, μ-opioid GPCRs inhibit presynaptic $Ca_V2.2$ channels and mediate intrathecal opioid analgesia [101–105]. We discovered that e37a, one of a pair of mutually exclusive exons the other being e37b, was expressed at higher levels in nociceptors compared to other neurons that primarily express e37b-containing $Ca_V2.2$ mRNAs [106]. We went on to show that $Ca_V2.2$ channels which contain e37a sequence exhibited different sensitivity to inhibition by GPCRs, including μ-opioid receptors, as compared to e37b-containing $Ca_V2.2$ isoforms [107]. By eliminating the contribution of e37a using either isoform-specific siRNAs or by removing e37a sequence from the *Cacna1b* mouse gene (replacing it with e37b; Fig. 4), we showed that e37a contributed to nociception and that it enhanced intrathecal morphine analgesia to thermal stimuli [108–110]. By contrast, ziconotide was equally effective at inhibiting e37a and e37b $Ca_V2.2$ splice isoforms and was an equally effective analgesic *in vivo* independent of e37a [109].

7. METHODS FOR STUDYING THE BEHAVIORAL IMPORTANCE OF ALTERNATIVELY SPLICED EXONS

There has been relatively slow progress in discerning the biological and pharmacological significance of cell-specific alternative splicing of *Cacna1* pre-mRNAs. This is more related to the technical challenges of separating the relative contributions of each Ca_V splice isoforms especially *in vivo*, and not the potential significance of their biological functions [2, 3]. Gene deletion and mutation studies in animal models, in conjunction with analyses of disease-causing mutations in human *CACNA1* genes, have been essential in determining the biological functions of different *Cacna1* genes [10, 111–117]. Interestingly, rare mutations in either one of a pair of alternatively spliced exons in *CACNA1C* (e8 or e8a) underlie the devastating Timothy Syndromes characterized by severe multi-organ disorders including lethal arrhythmias, webbing of fingers and toes, congenital heart disease, immune deficiency, intermittent hypoglycemia, cognitive abnormalities, and autism [86, 118, 119]. The different clinical manifestations of Type I and Type II Timothy Syndrome are correlated with the exon of *CACNA1C*, e8 or e8a, which carries the gain-of-function mutation [118]. Two strategies have been used to delineate the functional and behavioral consequences of

different $Ca_V2.2$ splice isoforms *in vivo*: i) isoform-specific siRNA to achieve acute knockdown of a specific Ca_V protein [110]; and ii) eliminating one of a pair of alternative exons in the *Cacna1b* mouse gene generating novel mouse strains (Fig. 4) [108, 109]. The latter approach has the advantage of forcing inclusion of a particular exon (e37a or e37b) during splicing without disrupting intron sequence, and in the case of the e37b-only mouse, without affecting total $Ca_V2.2$ protein levels. We utilized e37b-only and wild-type mouse strains to extract the contribution of $Ca_V2.2$-e37a splice isoform in an otherwise normal background (wild-type levels of $Ca_V2.2$ current and protein expression, and no compensatory changes in non-$Ca_V2.2$ current or $Ca_V2.1$ protein) [108, 109, 120]. By comparing behavioral phenotypes we found evidence that intrathecal morphine analgesia to noxious thermal stimuli was reduced in mice lacking e37a (e37b-only) as compared to wild-type mouse strains [108, 109]. Similar analyses of other alternatively spliced exons should help establish their biological functions.

CONCLUSION

Voltage-gated calcium ion channels are essential for mediating depolarization-induced calcium entry into excitable cells, and they are important drug targets for treating several disorders including those of cardiovascular and nervous systems. Human Ca_V channel clones are routinely used to screening for new drugs to inhibit, or activate, calcium channels for use in humans. Even a single amino acid difference between human and non-human Ca_V proteins could affect drug efficacy. The amino acid composition of discrete regions of Ca_V channels, originating from cell-specific alternative splicing, can vary substantially across isoforms in different tissues. Intensive transcriptome sequencing projects for a range of human tissues are allowing quantification of expression patterns for each gene, including *CACNA1* genes, as well as alternative exon usage according to tissue-type, age and disease state. Cell-specific alternative splicing can modify the pharmacological sensitivities of Ca_V channels to drugs, toxins and G protein coupled receptors. Knowledge of expression patterns of Ca_V isoforms in different human tissues and disease states should inform strategies aimed at developing the next generation of Ca_V channel inhibitors and agonists with improved tissue-specificity.

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Biography

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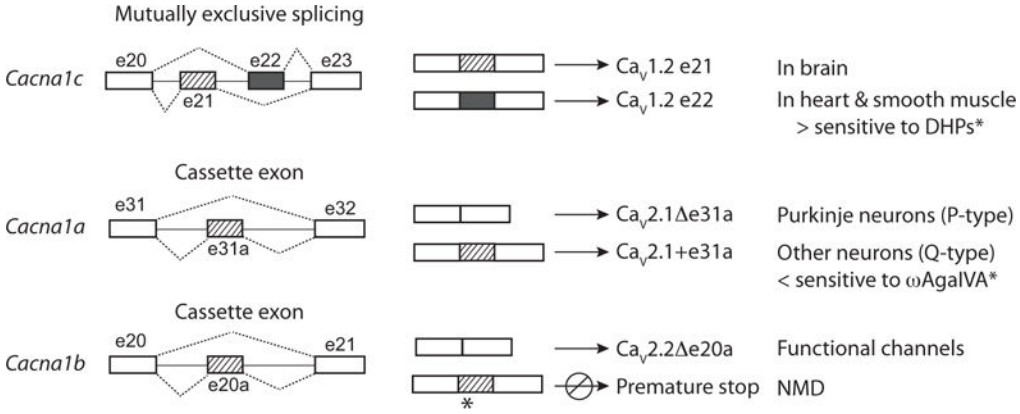

Fig. (1).

Examples of alternative pre-mRNA splicing that have been observed for *Cacna1* genes. Alternative splicing can involve the selection of one of a pair of mutually exclusive exons that are the same (or similar) lengths but that encode slightly different amino acid sequences. A pair of mutually exclusive exons, e21 and e22, are present in *Cacna1c*. E21 is selected more frequently during pre-mRNA splicing in brain, whereas in heart and smooth muscle, e22 is selected more often (see Table 1) [85]. E21 and e22 influence the sensitivity of $Ca_V1.2$ currents to DHPs [9, 77, 78, 85]. The inclusion of one mutually exclusive exon is often required to maintain the correct reading frame, and if neither or both are included during splicing a premature stop codon is often introduced resulting in NMD. Cassette exons are included or excluded during alternative pre-mRNA splicing. E31a of *Cacna1a* is excluded during pre-mRNA splicing in Purkinje neurons of the cerebellum (P-type currents), whereas in other neurons including cerebellar granule cells, e31a is included (Q-type currents). The presence of e31a influences inhibition by ω-agatoxin IVA [61]. The cassette e20a in *Cacna1b* is repressed in most neurons but if included it introduces a premature stop codon (see GenBank *FJ609386.1*). Certain cells may use e20a to down regulate $Ca_V2.2$ protein expression levels.

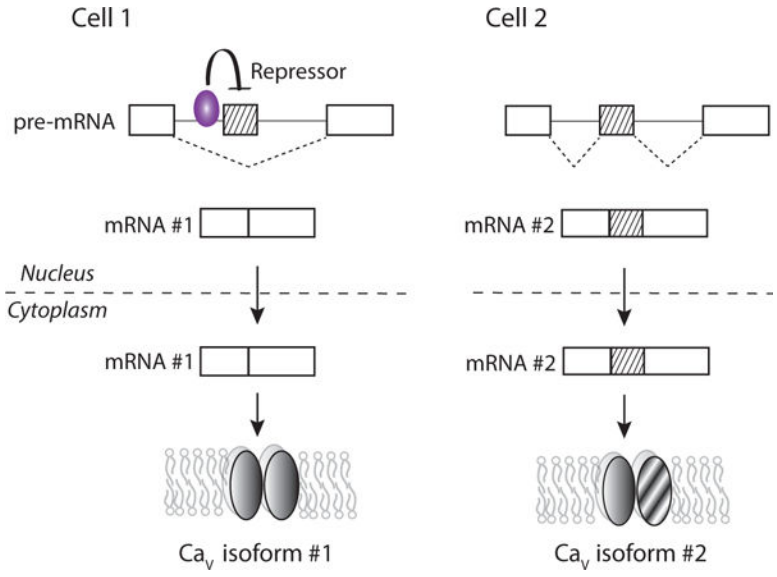

Fig. (2).

Cell-specific RNA binding proteins promote or repress exon inclusion during alternative splicing of pre-mRNAs. The example illustrates the difference in splicing patterns of the same region of pre-mRNA in Cell 1 that expresses a RNA binding splicing factor and in Cell 2 that does not. The splicing factor represses the inclusion of a cassette exon during alternative splicing of pre-mRNA. The splicing factor binds to specific nucleotide sequences upstream of the target exon in pre-mRNA. In cell 2, the absence of the splicing repressor results in exon inclusion during pre-mRNA splicing. The fully processed mRNAs are transported into the cytoplasm and are translated into different Ca_V splice isoforms. Certain RNA binding proteins including NOVA, rbFOX and PTB families can enhance or repress exon inclusion depending on where they bind (upstream or downstream) relative to the target exon [33, 40].

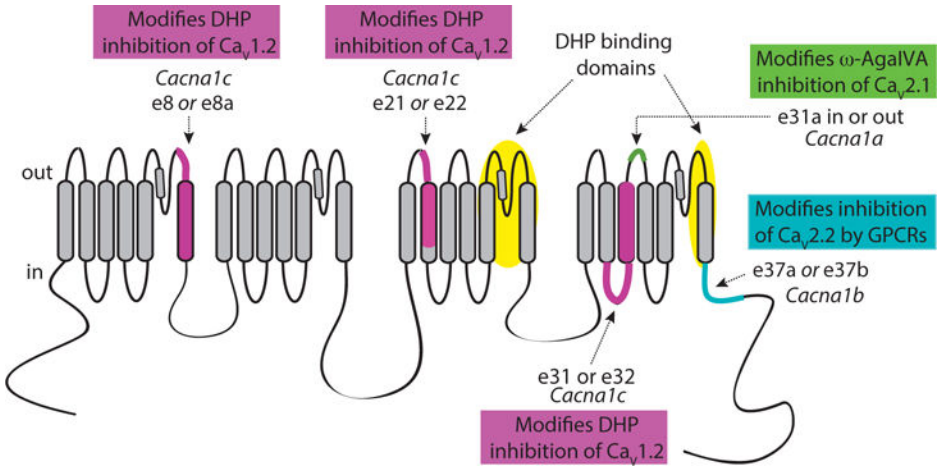

Fig. (3).

Schematic of major transmembrane spanning, intracellular, and extracellular domains of Ca_V channels as well as variable regions encoded by alternatively spliced exons that influence Ca_V channel pharmacology. Mutually exclusive exons e8a/e8, e21/e22 and e31/e32 of *Cacna1c* encode transmembrane spanning segments of $Ca_V1.2$ and they influence DHP inhibition by various mechanisms, including modifying voltage-dependent inhibition by DHPs [77, 78, 81, 84, 85]. Putative high-affinity DHP binding sites in $Ca_V1.2$ (shaded). Cassette e31a of *Cacna1a* encodes two amino acids asparagine proline (NP) in the extracellular linker IVS3–IVS4 of $Ca_V2.1$ and modifies inhibition by ω-agatoxin IVA [61]. Mutually exclusive e37a and e37b of *Cacna1b* encode 33 amino acids in the proximal C-terminus of $Ca_V2.2$ and they influence G protein coupled receptor mediated inhibition [107, 108].

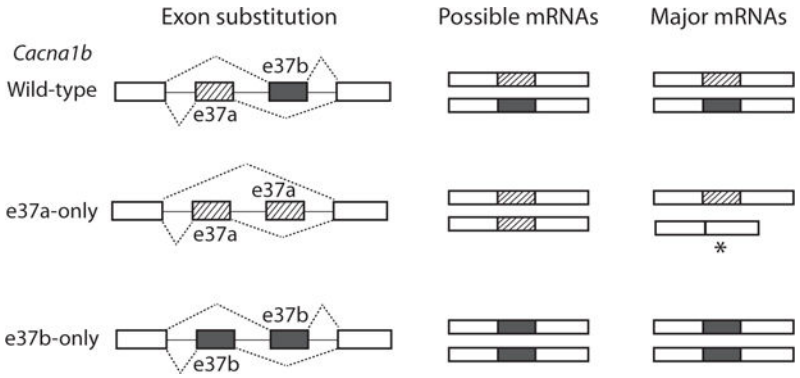

Fig. (4).

Exon substitution strategy employed to elucidate the functional and behavioral contributions of e37a and e37b of *Cacna1b*. Mutually exclusive e37a and e37b are the same length but about 30% of amino acids they encode differ. E37b was replaced by a second copy of e37a, in the e37a-only mouse strain, and e37a was replaced by a second copy of e37b, in the e37b-only mouse strain. E37a is repressed in most region of the nervous system but is found at higher levels in dorsal root ganglia, e37b is expressed throughout the nervous system. E37a does not substitute for e37b in the e37a-only mouse, there is increased e37 lacking $Ca_V2.2$ mRNA in neurons, and significantly reduced $Ca_V2.2$ protein levels. Whereas, in the e37b-only mouse, e37b is inserted in place of e37a and wild-type levels of $Ca_V2.2$ protein is preserved [108, 120].

Table 1

CACNA1 Genes, Proteins, Currents.

Gene name: human chromosome	Protein name	Exon, splicing factor	Current	Pharmacology (influenced by exon)
CACNA1S: chr 1	$Ca_V1.1$	e29, rbFox [121]	L	DHP– Isradipine and nisoldipine
CACNA1C: chr 12	$Ca_V1.2$	e8a, PTB [47] e9*, rbFox [48] e21/e22, ND e33, rbFox [48]	L	(e8/e8a; e21/22) [77,78,81,84,85] Diltiazem (e9*) [79]
CACNA1D: chr 3	$Ca_V1.3$	e8b, rbFox [121] e42a, ND	L	DHP – Nimodipine (e42a) [80]
CACNA1A: chr 19	$Ca_V2.1$	e24a, Nova(+) [49] e31a, Nova (−) [49]	P/Q	ω-Agatoxin IVA (e31a) [61]
CACNA1B: chr 9	$Ca_V2.2$	e24a, Nova(+) [122] e31a, Nova(−) [49] e18a, rbFox e37a/e37b, ND	N	μ-opioid and $GABA_B$ receptors - DAMGO, Morphine, Baclofen [107–109]

(+) indicates enhancer of exon inclusion and (−) repressor; ND = Not determined; All genes contain numerous sites of alternative splicing but only those for which the splicing factor has been determined, or that are known to influence drug sensitivity are listed here. For a more comprehensive review of alternative splicing in *Cacna1* genes see [2, 3, 15, 17, 31, 47–49, 61, 77–80, 91, 92, 108, 109, 121–124].