Review

Splicing for alternative structures of Cav1.2 Ca\(^{2+}\) channels in cardiac and smooth muscles

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Abstract

An estimate of up to 60% of genes are subjected to alternative splicing, and 15% of human genetic diseases are associated with mutation of the splice sites [Krawczak M, Reiss J, and Cooper DN. The mutational spectrum of single base-pair substitutions in mRNA splice junctions of human genes: causes and consequences. Hum Genet 1992; 90: 41–54; Cooper TA, and Mattox W. The regulation of splice-site selection, and its role in human disease. Am J Hum Genet 1997; 61: 259–66; Modrek B and Lee CJ. Alternative splicing in the human, mouse and rat genomes is associated with an increased frequency of exon creation and/or loss. Nat Genet 2003; 34: 177–80; Modrek B, Resch A, Grasso C, and Lee C. Genome-wide detection of alternative splicing in expressed sequences of human genes. Nucleic Acids Res 2001; 29: 2850–9; Lander ES, Linton LM, Birren B, Nusbaum C, Zody MC, Baldwin J, et al. Initial sequencing and analysis of the human genome. Nature 2001; 409: 860–921] [1–5]. The molecular diversity of alternatively spliced transcripts provides templates for a myriad of protein structures that are potentially crucial to sustaining the complexity of human physiology. The extensive alternative splicing of the \(\alpha\)1.2-subunit of the L-type Cav1.2 channel, producing splice variants with distinct electrophysiological and pharmacological properties, would impact directly on the function of the cardiovascular system. Cell-selective expression of Cav1.2 channels containing a specific alternatively spliced exon increases the functional variations for specific cellular activities in response to changing physiological signals. However, the regulation or control of the \(\alpha\)1.2-subunit alternative splicing machinery is unknown, and the role of numerous splice variants expressed in a cell is a mystery. A systematic and concerted effort is required to determine all the possible combinations of alternatively spliced exons in \(\alpha\)1.2-subunits in smooth and cardiac muscles. This will provide useful information to monitor changes on the usage of the entire suite of alternatively spliced exons to help relate altered Cav1.2 channel function to physiology and disease.

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Keywords: Alternative splicing; Calcium channel; Cardiovascular

1. Introduction

Voltage-gated (Ca\(_v\)) Ca\(^{2+}\) channels play a critical and essential role in numerous physiological processes and they open in response to membrane depolarization to allow rapid entry of Ca\(^{2+}\) ions to initiate muscle excitation–contraction coupling, neurotransmitter release, gene expression or hormone secretion. The Ca\(_v\) channels are heterooligomeric protein complexes comprising a main pore-forming Ca\(_v\)\(\alpha\)1-subunit in association with auxiliary Ca\(_v\)\(\beta\), Ca\(_v\)\(\alpha\)\(\delta\) and/or Ca\(_v\)\(\gamma\)-subunit. The Ca\(_v\)\(\alpha\)1-subunit is composed of four membrane-spanning domains (I–IV), and each domain consists of 6 transmembrane segments (S1–S6). Besides the cytoplasmic N- and C-termini, joining the domains are intracellular regions comprising the loops
linking domain I–II, domain II–III and domain III–IV (Fig. 1).

The Cavα1 family comprises 10 genes, of which 4 genes encode the L-type calcium channels referred to as Cavα1.1, Cavα1.2, Cavα1.3 and Cavα1.4 channels [6]. L-type Ca²⁺ channels are distinguished from the other 6 Cav channels by their selective sensitivity to 1,4-dihydropyridines (DHPs), phenylalkylamines (PAAs) and benzothiazepines (BTZs) and by their characteristic slow inactivating I_Ba currents [7]. Recently, missense mutations in mutually exclusive exons 8 and 8a of the α1.2 were found to be associated with Timothy’s syndrome in which patients suffer from multi-organ failure [8,9]. This work highlighted the importance of knowing all possible alternatively spliced exons of α1.2 in the screening for genetic mutations in ion channelopathies. In this review, we will focus on the molecular diversity of Cav1.2 calcium channels generated by alternative splicing and the functional implications in the cardiovascular system. We will discuss the proteomic variations of alternatively spliced isoforms of the Cav1.2 channels and relate the diverse structures to the electrophysiological and pharmacological properties of channel functions.

2. General view

Cavα1.2 channels are expressed widely in the cardiovascular and nervous systems. In cardiac and arterial smooth muscle cells, they are the major calcium channels acting as conduits for Ca²⁺ to pass through from the extracellular space into the cytoplasm. It has been shown that rapid entry of Ca²⁺ through the Cavα1.2 channels elicited two opposing effects on channel properties. With the rise in local [Ca²⁺]c, the Cavα1.2 channels inactivate to inhibit channel opening, while a global rise in cytoplasmic Ca²⁺ concentration facilitates the Cavα1.2 channels to promote channel opening. Both regulatory processes are mediated by calmodulin which pre-associates with the channel and acts as the Ca²⁺-sensor [10–12].

The critical role Cavα1.2 channels play in cardiovascular function is demonstrated acutely by the embryonic lethality of the knock-out mouse in which the CACNA1C gene has been ablated [13]. Further support of the essential role of Cavα1.2 channels was demonstrated by the phenotype of the conditional knockout of Cavα1.2 channel expression in mouse smooth muscle. The mice developed severe hypotension and related smooth muscle dysfunction leading to death one month after Cavα1.2 channel expression was conditionally ablated in blood vessels [14].

The human α1.2 gene, CACNA1C, contains 55 exons, of which 19 exons are subjected to alternative splicing, generating potentially 2¹⁹ combinations [15,16]. The 40 splice variations identified in 12 splice loci, together with the detailed genomic analyses of the exon–intron boundaries to demonstrate the splicing mechanisms involved have been reported recently [15]. Here, the tissue distribution, pharmacology and disease-related utilization of alternatively spliced exons of α1.2-subunit are summarized in Table 1. The combinatorial arrangements of the α1.2 alternatively spliced exons can conceivably produce an enormous number of phenotypic splice variations [17]. Cell-specific expressions of alternatively spliced exons that underlie potential selective physiological outcomes have been reported [18,19]. Similarly, selective assemblies of alternatively spliced exons in

![Fig. 1. Schematic representation of the pore-forming α1.2-subunit of the voltage-gated calcium channel Cavα1.2. The α1.2-subunit is composed of 4 domains (I–IV), each consisting of six putative transmembrane segments (S1–S6). The S4 segment is the voltage sensor containing 3–5 positively charged residues. The re-entrant S5–S6 loop region lines the pore and serves as the selectivity filter. Joining the domains are the cytoplasmic I–II, II–III and III–IV loops. The exons encoding the entire α1.2-subunit are indicated numerically and the boundaries between exons are indicated by lines across the schematic diagram.](https://academic.oup.com/cardiovascres/article-abstract/68/2/197/298725/28-March-2019)
of the coronary arteries to improve myocardial oxygen. Some of them can reduce heart rate and myocardial contractility as well, thus decreasing oxygen demand. Whether calcium channel blockers can increase cardiovascular events remains controversial. Several mechanisms have been proposed to explain increased risk of myocardial infarction after treating hypertensive patients with calcium channel blockers. One proposed mechanism is that the negative inotropic (contraction) effect from calcium channel blockers may reduce cardiac output and coronary flow. Another possible explanation could be that some calcium channel blockers produce a “coronary steal” phenomenon by which the blood is shunted from the ischemic to the non-ischemic myocardium. All these effects may be a consequence of the non-differentiating specificity of the calcium channel blockers that target the Ca\textsubscript{1.2} channel splice variants in the heart and in blood vessels.

Although all three classes of drugs bind to the α\textsubscript{1.2}-subunit, the mechanisms of block by these organic compounds are different. Inhibition by PAAs and BTZs is ‘use-dependent’, requiring the Ca\textsubscript{1.2} channels to be open before blocking occurs. However, DHPs show little or no ‘use-dependent’ inhibition, and instead, they block Ca\textsubscript{1.2} channels in a state-dependent manner by binding preferentially to the inactivated channels. The binding of the DHPs to the channels is increased substantially when the membrane becomes increasingly depolarized. Previous studies demonstrated that IIIS\textsubscript{5}, IIIS\textsubscript{6} and IVS\textsubscript{6} determine the binding of DHPs to the α\textsubscript{1.2}-subunit while IIIS\textsubscript{6} and IVS\textsubscript{6} participate in the binding of PAAs and DTZs [28]. However, IS\textsubscript{6} segment, encoded by mutually exclusive exons 8/8a, was shown to determine the sensitivity of the Ca\textsubscript{1.2} channels to isradipine, and thus may also participate in the binding of DHPs to the α\textsubscript{1.2}-subunit [29]. Unlike IS\textsubscript{6}, the exons encoding IIIS\textsubscript{5}, IIIS\textsubscript{6} and IVS\textsubscript{6} are constitutive exons. Recently, the report on the DHP-sensitivity knock-in mutant mice (IIIS\textsubscript{5}; T1066Y mutation) showed that IIIS\textsubscript{5} also participated in inhibition of PAA on Ca\textsubscript{1.2} channels [30].

### Table 1

<table>
<thead>
<tr>
<th>Exon</th>
<th>Function and selective distribution of alternatively spliced exons of Ca\textsuperscript{2+} channel α\textsubscript{1.2}-subunit</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>Cardiac muscle</td>
<td>Biel et al. [68], Dai et al. [69], Pang et al. [70], Saada et al. [71]</td>
</tr>
<tr>
<td>1</td>
<td>Smooth muscle</td>
<td>Biel et al. [68], Pang et al. [70], Saada et al. [71]</td>
</tr>
<tr>
<td>8</td>
<td>Smooth muscle</td>
<td>Biel et al. [68], Welling et al. [29], Lacinova et al. [72]</td>
</tr>
<tr>
<td>8a</td>
<td>Cardiac muscle</td>
<td>Biel et al. [68], Hu and Marban [73], Welling et al. [29]</td>
</tr>
<tr>
<td>9*</td>
<td>Smooth muscle</td>
<td>Liao et al. [19], Biel et al. [68], Bielefeld [59]</td>
</tr>
<tr>
<td>21</td>
<td>Less sensitive to DHPs</td>
<td>Soldatov et al. [74], Zuhlke et al. [63]</td>
</tr>
<tr>
<td>22</td>
<td>More sensitive to DHPs</td>
<td>Soldatov et al. [74], Zuhlke et al. [63]</td>
</tr>
<tr>
<td>31</td>
<td>More sensitive to DHPs</td>
<td>Zuhlke et al. [63], Diebold et al. [61]</td>
</tr>
<tr>
<td>45*</td>
<td>Oxygen sensing</td>
<td>Fearon et al. [76]</td>
</tr>
</tbody>
</table>

Exons 1/1a, 8/8a, 21/22 and 31/32 are mutually exclusive exons. Exon 9* is included or excluded in different tissues. The sensitivity to DHPs is affected by different membrane holding potentials and different combinations of alternatively spliced exons. See references for the detailed information.

3. Calcium channel blockers

One important characteristic of useful calcium channel blockers is their ability to selectively inhibit the influx of calcium ions through Ca\textsubscript{\textit{a}} channels. L-type calcium channel blockers have been shown to be effective in controlling blood pressure and managing anginal symptoms. They are well tolerated and are associated with minimal side effects, such as headache, flushing, hypotension, pedal edema and are the most frequently prescribed antihypertensive drugs [25]. The major part of agonist-induced Ca\textsuperscript{2+} increase is from L-type Ca\textsuperscript{2+} channels, mainly Ca\textsubscript{1.2} channels, in vascular smooth muscle cells, which is important to maintain the basal tone of smooth muscles [26,27]. All calcium channel blockers lower arterial pressure by reducing peripheral vascular resistance resulting in vasodilation response to physiological or pathological triggers have also been described [20–24].

4. Ca\textsubscript{1.2} channels in cardiac and smooth muscles

The phenotypic diversity of α\textsubscript{1.2}-subunit arising from alternative splicing is especially relevant to arterial smooth muscle or cardiac muscle functions as there might not be a direct role for Ca\textsubscript{1.3} channels in both cardiac inotropy and smooth muscle contractility [31]. The α\textsubscript{1.2}-subunit of the L-type calcium channel may be customized in its proteomic structures to provide the necessary ability of the smooth muscle to contract at physiological potentials. In comparison to the analogous calcium channels found in heart, the voltage-dependence of activation for smooth muscle L-type channels exhibited a hyperpolarized shift of −15 mV [32,33]. There is a close correlation between activation potential of L-type channels and tension in the smooth muscles [32]. The more hyperpolarized activation properties
of the smooth muscle Cav1.2 channels are required for sufficient tension to be generated by modest physiological membrane depolarization. Only a small amount of tension would be generated by smooth muscle if the voltage-dependent activation potentials of smooth muscle L-type channels were similar to the cardiac/neuronal Cav1.2 channels. Nonetheless, the molecular basis for this specialization may be in part attributed to the inclusion of a specific alternatively spliced exon 9* in the I–II loop of α1.2-subunit [19].

Screening of cDNA libraries and analyses of the genotypic structures of the α1.2 clones identified the “smooth muscle” and “cardiac muscle” α1.2 splice variants to differ in at least the following 4 regions: (i) exon 1/1a; (ii) exon 8/8a; (iii) ±75 nt in I–II loop (termed exon 9* in [18]) and (iv) exon 31/32. The cardiac muscle form was proposed to consist of splice combination 1a/8a/–9*/31; while the proposed smooth muscle splice combination was 1/8/+9*/32. In rabbit heart, exclusion of either exon 19 or both exons 17 and 18 would translate the α1.2-subunit with a premature stop codon in the II–III loop region to form hemichannels [34]. Such hemichannels have also been predicted in the α2.1 and α2.2 channels where in the case of the α2.1 channels, the hemichannels played a dominant negative role by competing for the β-subunit [35–37]. On the other hand, the suppression of channel functional expression by hemichannels of the α2.2-subunit or truncated α1.2-subunit lacking the N-terminus was not via competition for the β-subunit but might be a direct effect on the full-length channels [36,38]. Notably, some mutations of the α2.1-subunit that were linked to familial episodic ataxia type 2 were predicted to produce truncated channels but the pathogenic mechanism is still unknown [39,40]. Interestingly, truncated or hemichannels have also been described for both K+ and Na+ channels in mammals and insects [41–43].

Through a PKC-mediated effect, the inhibition by exon 1a is relieved in exon 1a-containing α1.2-subunit in cardiac muscles [44,45]. The inclusion of either exon 8 or exon 8a regulates DHPs sensitivity. The “smooth muscle” form of α1.2-subunit contains exon 8 and exhibits larger inhibition by DHPs [29]. Based on the combination of exons mentioned above, several calcium antagonists (e.g., nifedipine, nisoldipine, and isradipine) bind with some selectivity to the ‘smooth muscle’ form of L-type calcium channel, whereas verapamil binds equally well to both ‘cardiac’ and ‘smooth muscle’ forms of the α1.2-subunit [46]. However, the effects of DHPs on Cav1.2 channels expressed in native cardiac and vascular tissues revealed a much higher ratio of IC50hheart/IC50vessel as compared to results obtained from in vitro studies, under similar membrane potentials [47–49]. This indicates that the actual combinations of calcium channels in cardiovascular system are far more complicated than the limited channel clones used in heterologous expression systems. Comprehensive future work is required to provide insights into the genetic differences that may exist between smooth and cardiac muscle α1.2-subunits in the entire suite of identified alternatively spliced exons [15].

Selective high expression of exon 9*-containing α1.2-subunit was identified in the smooth muscle layer of arteries, providing another piece of supporting evidence for the notion of restricted localization of an alternatively spliced exon for specific biological functions [18]. Exon 9* is located in the I–II loop of the α1.2, immediately downstream of the alpha-interacting domain (AID). The binding of the β-subunit to the AID modulates Cav1.2 channel function by enhancing Ca2+ currents and changing the kinetics of the activation and inactivation properties of the channel [50]. However, whether the addition of 25-aa encoded by exon 9* may define any binding selectivity of the different β-subunits remains to be tested. In the alternative splicing of the I–II loop of the α1.2-subunit, it was shown that different β-subunits did not affect the inherent differences in inactivation or current–voltage relationships between the α1A-a, and α1A-s splice variants [51]. On the other hand, alternative splicing of the β2-subunit determined α1-subunit-specific effects on channel biophysical properties and block by αv-conotoxin MVIIIC [52,53]. Similarly, alternative splicing of β2 generated 5 splice variants that displayed different effects on α1.2 channel inactivation kinetics and prepulse facilitation [54].

Protein kinase A (PKA) regulates cardiac and smooth muscle Cav1.2 channel functions to different levels [55], and the putative phosphorylation site has been identified at S1928 of the α1.2-subunit [56–58]. However, another putative PKA site may be located on exon 9* [59]. Interestingly, the threonine residue in exon 9* is only present and conserved in human and rabbit, but is absent in rat and mouse α1.2-subunits (GenBank Accession Number AY323810 and U17869). There is the possibility of species variation in PKA regulation of the Ca1.2 channels and this hypothesis requires further experiments.

As discussed earlier, alternative splicing of a single exon 9* appears to underlie the key shift in voltage activation potentials seen in cardiac and smooth muscle L-type calcium channels. The inclusion of exon 9*, however, did not affect the sensitivity of Cav1.2 channel splice variants to nifedipine blockade or voltage-dependent steady-state inactivation [19]. This report provided further evidence for phenotypic variation generated simply by employing cassette exons to produce functional diversity that impact significantly on cellular physiology of different tissues, in this case between smooth and cardiac muscles.

One possible role for the high expression of exon 9*-containing α1.2-subunit in smooth muscle of arterial wall is to allow the activation of the channels at slight depolarization of the membrane potential and generate enough tension for blood vessels to function. The more depolarized resting membrane potential of arterial smooth muscle may serve to facilitate the ease of activation of the exon 9*-containing Ca1.2 channels after sympathetic stimulation or elevation of intravascular pressure. The process would contribute to Ca2+...
influx that presumably triggers greater myogenic tone or more prolonged contraction of the blood vessels [60].

5. Alternative splicing of Ca_{1.2} channels and diseases

One splice locus that was demonstrated to switch in its level of expression in disease is the mutually exclusive exons 31/32 of the α_{1.2}-subunit [20]. The functional differences between the inclusion of exon 31 or exon 32 have not been elucidated yet by electrophysiology [15]. Nevertheless, the alternative splicing of exons 31/32 is regulated developmentally. In adult heart, it was reported that exon 31 was the major form while fetal heart contained more of exon 32 [61]. However, this result was not corroborated by a recent report that demonstrated that both adult and fetal heart predominantly expressed exon 32 [15]. It has, however, been shown that there was a switch in the levels of expression of exons 31/32 in human heart failure (Table 1) [20]. The physiological significance for such an alteration in the usage of alternatively spliced exons is still unknown.

Recently a de novo point mutation within exon 8a was identified in Timothy syndrome [8]. Multiple organ systems were affected, including heart, skin, eyes, teeth, immune system and brain. Interestingly, this de novo point mutation occurs mainly in exon 8a; this mutually exclusive exon encodes the IS6 transmembrane segment (Fig. 1). Electrophysiological characterization of the G406R mutant Ca_{1.2} channel showed an almost complete loss of voltage-dependent inactivation or a drastic slowing of Ca^{2+}-dependent inactivation. The prolonged Ca^{2+} current may account for severe arrhythmias, congenital heart disease, cognitive impairments, and mental retardation. In a related paper, Splawski et al. have also identified two missense mutations of exon 8 in two patients with severe Timothy’s syndrome in which the patients had severe prolongation of the QT interval. Both mutations gave rise to almost complete abolition of voltage-dependent inactivation of the human α_{1.2} channels. It was suggested that due to the high expression of exon 8 in human heart and brain, there was possibly a correlation between the mutations discovered in exon 8 to the severe symptoms seen in the patients [9]. It should be noted that the nomenclature used for the mutually exclusive exons 8 and 8a in the two Splawski’s papers on Timothy syndrome are opposite to those used in other reports [15,62,63]. Previous reports placed exon 8a before exon 8 in the human genomic sequence (GenBank Accession Number Z26263), and as such what were referred to as exons 8 or 8a in the Splawski’s papers are exons 8a or 8 respectively in the other reports.

6. Perspective

Some compelling questions on the role of alternative splicing of calcium channel in physiology await future research to provide the answers. The biological role of a particular alternatively spliced exon of the α_{1.2}-subunit is unknown. Nonetheless, the role of alternative splicing has been well characterized in sex determination in Drosophila, and in chick hair cell frequency tuning [64,65]. Another intriguing question is the identity of the predominant combinatorial assembly of alternatively spliced exons of the α_{1.2}-subunit in different cell- or tissue-types. Such information may provide more suitable targets for screening for therapeutics or for disease management. Equally fascinating is the understanding of what directs alternative splicing and what factors regulate the specific expression of alternatively spliced exons spatially or temporally, and in physiology or disease. Lastly, it is extremely puzzling why there is a need for such a great number of splice variants and why they are expressed and distributed in various ratios and combinations in different cells. It is challenging to be able to follow the changes in the splicing profiles dynamically and to deduce the physiological role for the alteration in combinatorial splicing patterns in biology or disease. It is speculative to suggest that alteration in the ratio of expression of a single and critical splice locus may have a great impact on normal physiological function and that shifting the ratio beyond a threshold value may lead to pathological consequences [66,67]. Nevertheless, it is evident that alternative splicing is an exquisite mechanism that operates to diversify Ca_{1.2} channel structures by the combinatorial inclusion or exclusion of alternatively spliced exons. The molecular diversity of α_{1.2}-subunit contributes to the numerous proteomic variations of the Ca_{1.2} channels that may fine-tune channel functions elegantly to specific cellular activities or in response to a variety of signals.

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