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Signature combinatorial splicing profiles of rat cardiac- and smooth-muscle $Ca_v1.2$ channels with distinct biophysical properties

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Abstract

L-type ($Ca_v1.2$) voltage-gated calcium channels play an essential role in muscle contraction in the cardiovascular system. Alternative splicing of the pore-forming $Ca_v1.2$ subunit provides potent means to enrich the functional diversity of the channels. There are 11 alternatively spliced exons identified in rat $Ca_v1.2$ gene and random rearrangements may generate up to hundreds of combinatorial splicing profiles. Due to such complexity, the real combinatorial splicing profiles of $Ca_v1.2$ have not been solved. This study investigated whether the 11 alternatively spliced exons are spliced randomly or linked and if linked, how many combinatorial splicing profiles can be arranged in cardiac- and smooth-muscle cells. By examining three full-length cDNA libraries of the $Ca_v1.2$ transcripts isolated from rat heart and aorta, our results showed that the arrangements of some of the alternatively spliced exons are tissue-specific and tightly linked, giving rise to only 41 alternative combinatorial profiles, of which 29 have not been reported. Interestingly, the 41 combinatorial profiles were distinctively distributed in the three $Ca_v1.2$ libraries and the one named "heart 1–50" contained unexpected splice variants. Significantly, the tissue-specific cardiac- and smooth-muscle combinatorial splicing profiles of $Ca_v1.2$ channels demonstrated distinct electrophysiological properties that may help rationalize the differences observed in native currents. The unique sequences in these tissue-specific splice variants may provide the potential targets for drug design and screening.

Keywords: Calcium channel; L-type calcium channel; $Ca_v1.2$; Alternative splicing; Splice variant

1. Introduction

The $Ca_v1.2$ L-type voltage-gated calcium channels play critical roles in membrane excitability, gene expression, cardiac- and smooth-muscle contraction [1–4]. The $Ca_v1.2$ channel is a complex of three distinct subunits, the pore-forming α_1 (or $Ca_v1.2$) subunit and an intracellular β subunit and a transmembrane disulfide-linked $\alpha_2\delta$ subunit [5]. The $Ca_v1.2$ subunit of ~240 kDa is the largest subunit and it incorporates the conduction pore, the voltage sensor and gating apparatus and sites for channel regulation by second messengers, drugs and toxins [6,7]. Interestingly, the $Ca_v1.2$ subunit is subjected to extensive alternative splicing and generates many isoforms with distinct functional diversity. There are 10 among the 52 known rat $Ca_v1.2$ exons that undergo alternative splicing [8–13]. These exons are the mutually exclusive exons 1/1a, 8/8a, 21/22, 31/32 and the alternate exons 9* and 33 (Fig. 1). In this study, we identified another alternate exon 32-6nt which is generated by splicing at alternate donor site of exon 32 resulting in the deletion of last six nucleotides, while the human counterpart of this splice variation has been identified [14,15]. Previous studies have shown that exon 1a is subjected to protein kinase C regulation [13,16,17], exon 8/8a and exon 21/22 confer different pharmacology to channels [18–20] and $Ca_v1.2$ subunits containing exon 9* or exon 33 alter channel gating properties [15,21].

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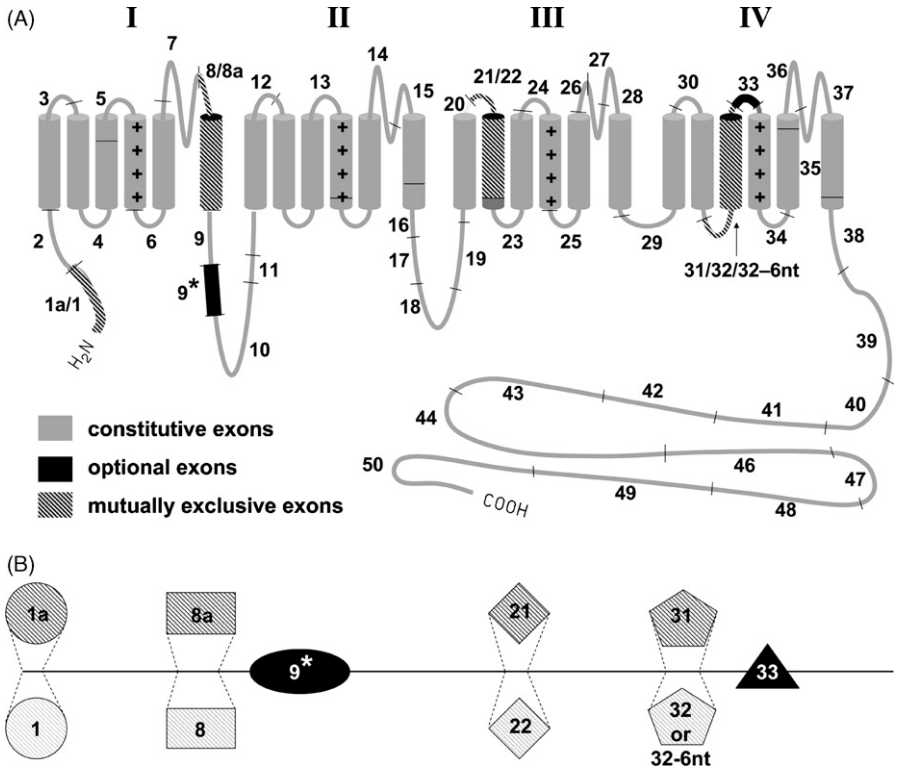

Fig. 1. (A) Diagrammatic representation of 11 alternatively spliced exons of the rat $Ca_v1.2$ subunit. There are two optional exons indicated by black boxes (9* and 33) and eight mutually exclusive exons indicated by hatched boxes (1, 1a, 8, 8a, 21, 22, 31 and 32). In addition, exon 32 can be spliced out entirely as an exon or at alternate donor sites resulting in the deletion of last six nucleotides (32-6nt). In total, 52 exons (exon 1 to 44, exon 46–50 and exon 1a, 8a and 9*) have been identified in rat. The numbering of exons is followed the numbering applied in annotating human $Ca_v1.2$ subunit [15,51]. (B) The relative positions of 11 alternative exons in full-length rat $Ca_v1.2$ transcripts.

Theoretically, these 11 alternatively spliced exons might generate a large number of, i.e. $2 \times 3 \times 2^7 = 768$ different exon-combinations of $Ca_v1.2$ transcripts. Here, the estimated number is calculated according to the proposition that there are two alternative initial exon 1a and exon 1 (also referred to as exon 1b) driven by the two distinct promoters [22–24], three alternative options of exon 32, exon 32-6nt or (-exon 32) and the other seven alternatively spliced exons 8a, 8, 9*, 21, 22, 31 and 33. It is noteworthy that the putative mutually exclusive exons 8a/8, 21/22, 31/32 are not strictly mutually exclusive as shown in this study and in previous reports [14,15]. Due to such complexity, the real combinations of alternatively spliced exons of $Ca_v1.2$ gene in native tissues have not been solved, leaving a critical gap in knowledge in the $Ca_v1.2$ channel field. Therefore, the existence of these 11 alternatively spliced exons poses challenging questions: (1) Are the combinatorial arrangements of the alternatively spliced exons random or linked? (2) If linked, how many combinatorial splicing profiles are there in muscle tissues? And (3) are there tissue-specificity in the expression of the $Ca_v1.2$ splicing profiles?

This study attempts to provide answers to these questions by generating three full-length $Ca_v1.2$ cDNA libraries: one specific to aorta smooth muscle and two heart muscle libraries containing either exon 1 or exon 1a, the alternative initial exons. To circumvent the limitation of traditional cDNA library screening in which the desired cDNA clones are generally fragmented and are presented in very limited copy numbers, we employed long RT-PCR method to generate 284 full-length $Ca_v1.2$ cDNA clones in the three libraries. The utilization of alternatively spliced exons was examined by PCR colony screening in 96-well format for each of the 11 exons. Our results showed a complexity in combinatorial splicing profiles that surpassed what is known of two previously reported $Ca_v1.2$ splice variants, namely the "cardiac form" $\alpha_{1C\text{-}a}$ (1a, 8a, −9*, 31) and "smooth muscle form" $\alpha_{1C\text{-}b}$ (1, 8, 9*, 32) [6,19,25–28]. Our study found 41 full-length $Ca_v1.2$ transcripts in heart and aortic tissues of Wistar Kyoto (WKY) rats with a few signature splicing profiles to describe cardiac- or smooth-muscle $Ca_v1.2$ channels. Our data also indicated that expression of alternatively spliced exons are linked and some individual exons have tissue-specific expression, while in combination, distinct splicing profiles can be identified in cardiac- and smooth-muscles. These various $Ca_v1.2$ combinatorial splice variants exhibited phenotypic variations of electrophysiological properties which as a whole support the importance of alternative splicing in generating channel functional diversity in muscle.