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Neuronal calcium channels: Splicing for optimal performance

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Abstract

Calcium ion channels coordinate an astounding number of cellular functions. Surprisingly, only 10 $Ca_V\alpha_1$ subunit genes encode the structural cores of all voltage-gated calcium channels. What mechanisms exist to modify the structure of calcium channels and optimize their coupling to the rich spectrum of cellular functions? Growing evidence points to the contribution of post-translational alternative processing of calcium channel RNA as the main mechanism for expanding the functional potential of this important gene family. Alternative splicing of RNA is essential during neuronal development where fine adjustments in protein signaling promote and inhibit cell–cell interactions and underlie axonal guidance. However, attributing a specific functional role to an individual splice isoform or splice site has been difficult. In this regard, studies of ion channels are advantageous because their function can be monitored with precision, allowing even subtle changes in channel activity to be detected. Such studies are especially insightful when coupled with information about isoform expression patterns and cellular localization.

In this paper, we focus on two sites of alternative splicing in the N-type calcium channel $Ca_V2.2$ gene. We first describe cassette exon 18a that encodes a 21 amino acid segment in the II–III intracellular loop region of $Ca_V2.2$. Here, we show that e18a is upregulated in the nervous system during development. We discuss these new data in light of our previous reports showing that e18a protects the N-type channel from cumulative inactivation. Second, we discuss our published data on exons e37a and e37b, which encode 32 amino acids in the intracellular C-terminus of $Ca_V2.2$. These exons are expressed in a mutually exclusive manner. Exon e37a-containing $Ca_V2.2$ mRNAs and their resultant channels express at higher density in dorsal root ganglia and, as we showed recently, e37a increases N-type channel sensitivity to G-protein-mediated inhibition, as compared to generic e37b-containing N-type channels.

Keywords: Calcium channel; N-type; $Ca_V2.2$; Nociception; Development; Alternative splicing; Modulation; Opioids; GABA; Synprint

1. Alternative splicing in voltage-gated calcium channels

Human, rat, and mouse genomes contain 10 genes that encode $Ca_V\alpha_1$ subunits. The nervous system expresses nine of these genes. $Ca_V\alpha_1$ genes are large, spanning 100–800 kb of human genome sequence and containing upwards of 50 exons [1–4]. The large size of these genes provides ample opportunity for alternative splicing. Based on already known splice sites, the theoretical number of splice isoforms possible from a single $Ca_V\alpha_1$ subunit exceeds 1000 [3,4]. Other genes, far smaller than $Ca_V\alpha_1$ genes, have the potential to generate a similar or greater number of splice isoforms [5], and in some genes the number of possible isoforms is in the tens of thousands [6].

Alternative splicing occurs at sites important for controlling calcium channel activity, creating an array of functionally distinct calcium channels. Thus each neuron, in theory, can optimize calcium channel activity for the specific task at hand by orchestrating appropriate exon selection. Placing a specific channel isoform within a specific cell type can in turn provide information about the requirements of particular calcium signaling cascades. Further, studies of alternative splicing can

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and have revealed the location of key domains on calcium channels that regulate their activity.

Investigators have shown that structural changes generated by alternative splicing can affect channel biophysics, density, targeting, post-translational modification, and coupling to downstream signaling pathways. Functional sites of alternative splicing involve regulation that depends on tissue type, cell type, stage of development, and neuronal

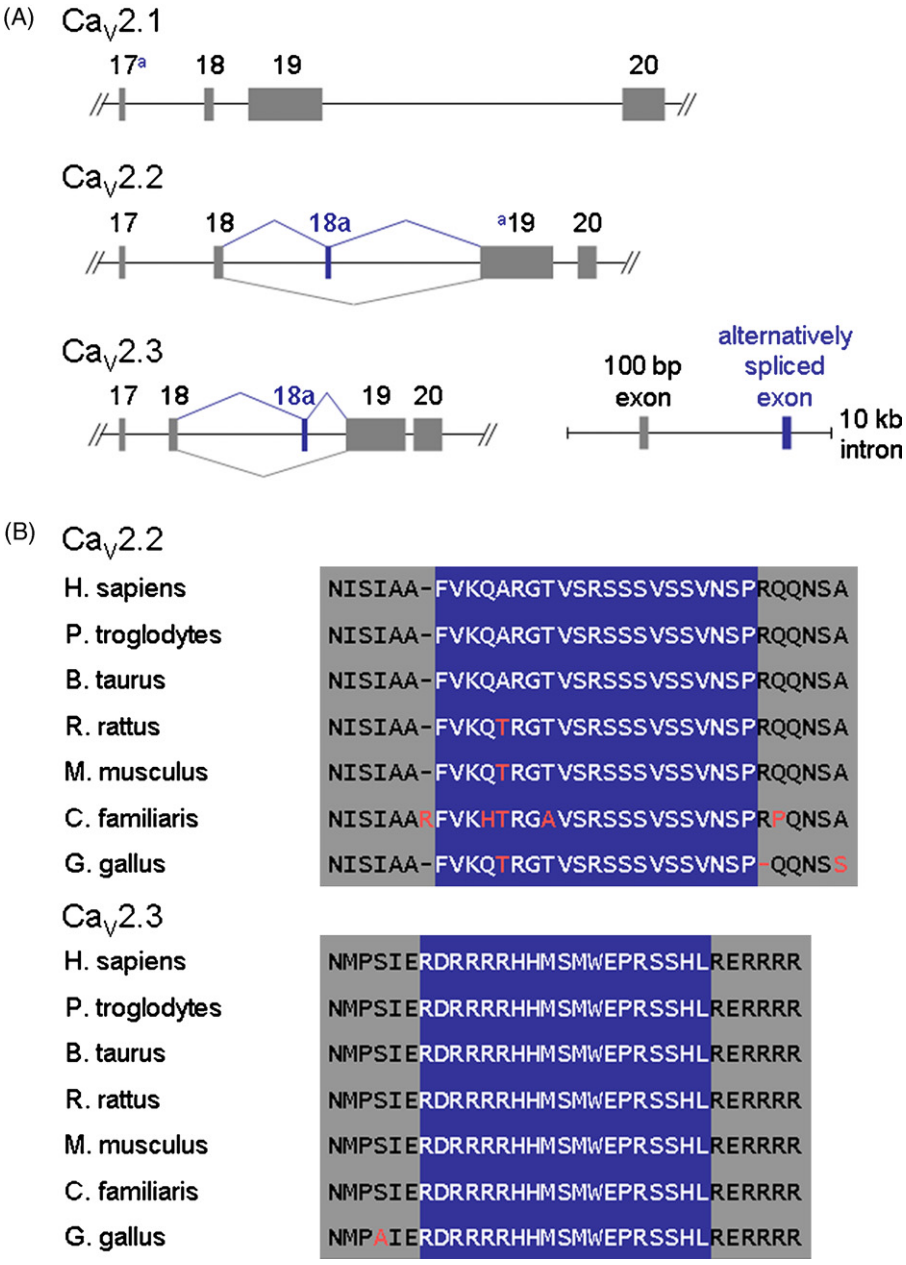

Fig. 1. (A) Alternatively spliced cassette exons, e18a, are found within human $Ca_V2.2$ and $Ca_V2.3$ genes. Partial gene structures, spanning e17 through e20, of human $Ca_V2.1$, $Ca_V2.2$, and $Ca_V2.3$ are illustrated. Exons are illustrated as gray (constitutive exons) or blue (alternatively spliced exons, e18a of $Ca_V2.2$ and $Ca_V2.3$) boxes, and introns are depicted as lines. Alternate 5′ donor ($Ca_V2.1$ e17) and 3′ acceptor ($Ca_V2.2$ e19) sites are indicated by superscript 'a'. We performed BLAST searches of the publicly available human genome sequence with published mRNA sequences for $Ca_V2.1$ (GenBank accession no. X99897), $Ca_V2.2$ (GenBank accession no. M94172, GenBank accession no. AF222338), and $Ca_V2.3$ (GenBank accession no. L27745). Intron and exon sizes are drawn to scale, as indicated. (B) Partial alignments of the deduced amino acid sequence of $Ca_V2.2$ (top) and $Ca_V2.3$ (bottom), including the alternatively spliced exon, e18a (blue and white), show limited variability across species. Abutting sequence of e18 and e19 (gray and black) are also included. $Ca_V2.2$ gene sequences were identified from the following depositions: *P. troglodytes*, GenBank accession no. NW_111847; *B. taurus*, GenBank accession no. NW_001024540; *C. familiaris*, GenBank accession no. NW_876333; *R. rattus*, GenBank accession no. NW_047651; *M. musculus*, GenBank accession no. NT_039206; *G. gallus*, GenBank accession no. NC_006104. $Ca_V2.3$ genes were found in the following sequence depositions: *P. troglodytes*, GenBank accession no. NW_101951; *B. taurus*, GenBank accession no. NW_972257; *C. familiaris*, GenBank accession no. NW_876323; *R. rattus*, GenBank accession no. NW_047398; *M. musculus*, GenBank accession no. NT_039184; *G. gallus*, GenBank accession no. NC_006095.

activity [7–22]. Importantly, the two sites of alternative splicing we discuss here, in the II–III intracellular loop region (e18a) and in the C-terminus (e37a and e37b), are conserved across a number of mammalian $Ca_V2.2$ genes, including human, rat, and mouse (see Fig. 1 for e18a sequence comparisons and see Ref. [7] for e37a and e37b comparisons). We reason that such sites of alternative splicing, that are under cellular control and that are evolutionarily conserved, must be located in key domains essential for regulating N-type channel function and likely evolved to contribute functional advantage to the organisms in which they occur [23,24].

2. The $Ca_V2.2$ N-type calcium channel

Our interest in the N-type calcium channel stems from its established role in the control of transmitter release from many different types of neurons [25,26]. The N-type calcium channel is also implicated in synaptogenesis and regulation of gene expression [27,28]. N-type calcium channels differ functionally across cell types and sub-cellular compartments within a given cell, suggestive of molecular and structural heterogeneity. Differences in N-type channel inactivation, single-channel conductance, gating, sensitivity to G-protein-mediated modulation, and ability to couple to transmitter release have been documented [29–39]. Several factors support functional diversity among voltage-gated calcium channels, including association with different auxiliary subunits, post-translational modifications, and association with target proteins and signaling molecules [40–47]. These processes are also controlled by alternative splicing [2,3,47–50].

3. Cassette exons in the II–III loops of Ca_V2 channels

The intracellular loops connecting homologous domains II and III ($L_{II–III}$) of pore-forming $Ca_V\alpha_1$ subunits are of special interest. This region supports key cellular functions and links calcium channels to downstream effector proteins. In skeletal muscle, the $L_{II–III}$ of $Ca_V1.1$ binds directly to ryanodine receptors on the sarcoplasmic reticulum, an interaction necessary and sufficient for excitation-contraction coupling [51–53]. In the case of Ca_V2 calcium channels, II–III loops connect channels to sites of synaptic vesicle fusion via binding to SNARE (soluble *N*-ethyl-maleimide-sensitive fusion (NSF) attachment protein receptor) proteins [54], critical for coupling N-type and P/Q-type calcium channels to transmitter release and for targeting channels to presynaptic nerve terminals [48,54–61]. The $L_{II–III}$ region of $Ca_V2.2$ channels also regulates cumulative inactivation of channels in response to stimulus trains [54,62,63].

Isoforms of $Ca_V2.1$, $Ca_V2.2$, and $Ca_V2.3$ exist that differ within the $L_{II–III}$ [16,64] and differ in their abilities to bind presynaptic SNARE proteins [48]. Our group has focused on cassette exons, e18a, located between exons 18 and 19 in $L_{II–III}$ of mammalian $Ca_V2.2$ and $Ca_V2.3$ genes that, as described below, are evolutionary conserved (Fig. 1). We showed previously that e18a in $Ca_V2.2$ protects the channel from inactivation. Specifically, e18a-containing $Ca_V2.2$ channels are less sensitive to closed-state inactivation, and therefore calcium influx during action potential trains is expected to be sustained relative to that through $Ca_V2.2$ channels lacking e18a [62].

3.1. Human Ca_V2 genomic sequences

Our interest in e18a was motivated from our analyses of various Ca_V2 gene sequences. Mammalian $Ca_V2.2$ and $Ca_V2.3$, but not $Ca_V2.1$, genomic sequences contain putative cassette exons between constitutive exons, e18 and e19. In rat, e18a resides within a large 6125 bp intron between e18 and e19 of the $Ca_V2.2$ gene [15]. We found a nearly identical cassette exon (61 of 63 bp), flanked by consensus gt–ag splice junctions, within the 9775 bp intron between e18 and e19 of the human $Ca_V2.2$ gene (see Fig. 1) [3]. We also identified the location of a 57 nucleotide exon within a 6221 bp intron of the human $Ca_V2.3$ gene, consistent with previous studies of $Ca_V2.3$ mRNAs [65,66]. Despite similarity in size and position, exons 18a of $Ca_V2.2$ and $Ca_V2.3$ contain distinct nucleotide sequences with only 25% homology and encode dissimilar amino acid sequences (Fig. 1B). A similar analysis of the $Ca_V2.1$ human gene revealed a much smaller intron (~1 kb) between e18 and e19, and no evidence for a putative cassette exon.

We also examined the genomic sequences of $Ca_V2.2$ and $Ca_V2.3$ for *Pan troglodytes*, *Bos taurus*, *Mus musculus*, *Canis familiaris*, and *Gallus gallus*. All these species contain alternatively expressed e18a of $Ca_V2.2$ and $Ca_V2.3$ (Fig. 1B), and exon sequences were highly conserved. By our analysis intracellular regions of the channels, including the C-terminus and loops connecting homologous domains, were $71.8 \pm 3.0\%$ similar among species. In comparison, $Ca_V2.2$ e18a nucleotide sequences shared $96.2 \pm 0.5\%$ similarity among the seven species investigated here, significantly greater than the average sequence conservation within constitutively expressed exons that encode intracellular domains of the channel ($p < 0.0001$).

3.2. Expression of $Ca_V2.2$ and $Ca_V2.3$ alternatively spliced exons in the human nervous system

We next used RT-PCR to establish that e18a of $Ca_V2.2$ and $Ca_V2.3$ are expressed in human neuronal tissue, and we also confirmed the lack of alternative splicing in the same region of $Ca_V2.1$ (Fig. 2A). We found $Ca_V2.2$ e18a expression in human whole brain and spinal cord (Fig. 2A), contrary to reports that this exon is not expressed in human cells [12]. Notably, the proportion of $Ca_V2.2$ e18a-containing mRNAs was higher in adult spinal cord as compared to whole brain. Further, $Ca_V2.2$ e18a was not detected in fetal whole brain.

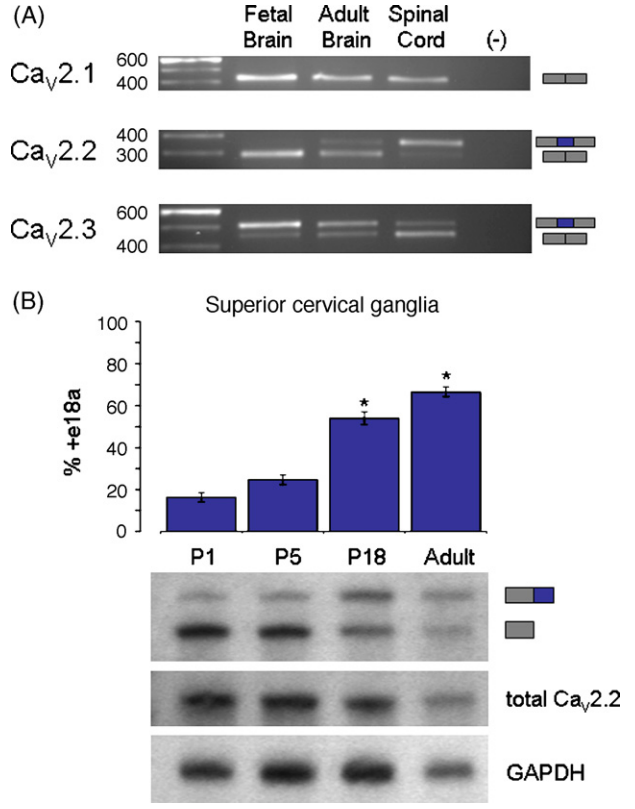

Fig. 2. (A) Regulated alternative splicing of $Ca_V2.2$ and $Ca_V2.3$ e18a occurs in the human central nervous system. Using primers that flank e18a, we performed RT-PCR for $Ca_V2.1$, $Ca_V2.2$, and $Ca_V2.3$ with RNA preparations from human fetal and adult brain as well as human adult spinal cord. RT-PCR of $Ca_V2.1$ (top) in this region results in one product of the appropriate size (455 bp). $Ca_V2.2$ primers (middle) produced a single product from fetal brain, compared to two products that differ by 63 bps from adult brain and spinal cord (+e18a, 351 bp; Δe18a, 288 bp). Two products (+e18a, 508 bp; Δe18a, 451 bp) result from the $Ca_V2.3$ PCR (bottom) for all three RNA sources. (B) $Ca_V2.2$ e18a expression increases during the first three postnatal weeks in rat SCG. We examined the developmental expression of e18a by RNase Protection Assay with the RPA III Kit (Ambion) and biotinylated antisense probes. (Top), Histogram indicates the percent of e18a-containing mRNA (upper band) as compared to the total signal from protected fragments for P1, P5, P18, and adult SCG RNA. Compared to P1 and P5 SCG, P18 and Adult SCG contain significantly greater proportions of e18a-containing $Ca_V2.2$ mRNA ($p < 0.002$). (Bottom), Representative gels showing typical results for three probes: (top) A probe designed to examine alternative splicing of e18a (240 min exposure), (middle) pan-$Ca_V2.2$ probe (180 min exposure), (bottom) GAPDH probe (10 min exposure). In the upper gel, the fully protected product contains e18a (381 bp; gray and blue bar), and the partially protected product (lower band) lacks e18a (318 bp; gray bar). GAPDH and pan-$Ca_V2.2$ probes were hybridized to SCG RNA in the same RPA reaction. P18 and adult $Ca_V2.2$ expression levels normalized to GAPDH are lower than those at P1 ($Ca_V2.2$: GAPDH ratios: P1 = 0.71 ± 0.06, P5 = 0.61 ± 0.10, P18 = 0.37 ± 0.09, adult = 0.30 ± 0.07, mean ± S.E.; $p < 0.05$).

Thus, $Ca_V2.2$ e18a expression in human is both regionally and developmentally regulated.

$Ca_V2.3$ e18a is also alternatively spliced in human tissues (Fig. 2A) [66]. Interestingly, as $Ca_V2.2$ e18a expression increased during development, there appeared to be a concomitant decrease in $Ca_V2.3$ e18a expression. Inclusion of e18a in $Ca_V2.2$ results in N-type calcium channels that are less sensitive to closed-state inactivation [62]. In contrast, $Ca_V2.3$ channels that contain e18a exhibit increased sensitivity to calcium-dependent current enhancement [67,68] and stimulation by phorbol esters [69].

3.3. Developmental regulation of $Ca_V2.2$ e18a

We next examined the developmental expression of $Ca_V2.2$ e18a in the rat superior cervical ganglia (SCG), a tissue that expresses relatively high levels of $Ca_V2.2$ and that contains a relatively homogenous class of neuron (Fig. 2B) [15]. We quantified e18a splice isoforms by ribonuclease protection from P1, P5, P18, and adult SCG. We used an antisense probe that was complementary to constitutively expressed exons 15–18 and the alternatively spliced e18a. $Ca_V2.2$ mRNAs that contain e18a fully protected the probe (Fig. 2B, top gel, upper band), while $Ca_V2.2$ mRNAs that lack the alternatively spliced exon yielded a partially protected probe (Fig. 2B, top gel, lower band). In the adult SCG, e18a was present in 67 ± 4% of all $Ca_V2.2$ mRNAs (Fig. 2B). By comparison, the relative abundance of e18a was 54 ± 5% at P18, 25 ± 4% at P5, and 16 ± 4% at P1 ($n = 3$ separate experiments for all ages). The adult expression level of e18a is significantly different than that in P1 and P5 SCG ($P < 0.0003$), and the proportion of e18a mRNA transcripts increased >4-fold between P1 and adulthood. The overall expression level of $Ca_V2.2$ mRNA decreased between P1 and P18, in comparison to GAPDH expression levels (Fig. 2B, bottom gels). This is consistent with an increase in the number of non-neuronal cells in the maturing SCG.

During the first three weeks of postnatal life, while e18a expression continuously increased in our assay (Fig. 2), SCG neurons undergo a variety of developmental changes. By birth, the majority of SCG neurons have differentiated after migrating into the ganglia, and soon after they begin to form synaptic connections with target structures. The vast majority of these connections have formed by P21 [70]. Rat SCG neurons rely exclusively on the $Ca_V2.2$ N-type calcium channel to control transmitter release [71]. Our data, therefore, indicate that $Ca_V2.2$ channels that contain the 21 amino acids encoded by e18a likely control transmitter release from mature SCG terminals and are specifically upregulated during the period of exuberant ganglion cell synaptogenesis for this purpose.

We note that alternatively spliced exons e18a within $Ca_V2.2$ and $Ca_V2.3$ show a high level of amino acid sequence conservation across mammalian and avian species (Fig. 1), when compared to constitutively expressed exons that encode intracellular regions of these channels. Others have also reported an exon homologous to mammalian e18a in the pufferfish $Ca_V2.2$ ortholog [72]. These data signify that additional evolutionary pressure exists to preserve e18a sequences and suggest an important functional role for $Ca_V2.2$ and $Ca_V2.3$ isoforms that contain the additional 21 and 19 amino acids encoded by their respective exons. Interestingly, our

data point to the possibility that a common mechanism regulates expression of e18a in $Ca_V2.2$ and $Ca_V2.3$ based on their approximate reciprocal expression patterns. Specifically, we find that e18a containing $Ca_V2.2$ mRNAs are more abundant in the mature brain (Fig. 1), peripheral ganglia [15], and spinal cord (Fig. 1), whereas, $Ca_V2.3$ mRNAs containing e18a are more abundant in fetal brain (Fig. 1). Further, $Ca_V2.3$ e18a mRNAs are virtually absent from the peripheral nervous system (unpublished observations, ACG and DL). One tantalizing possibility is that e18a controls targeting of $Ca_V2.2$ and $Ca_V2.3$ channels to sites of transmitter release, given that these exons modify amino acid composition in the II–III linker region. Although e18a is absent from $Ca_V2.1$, others have reported distinct $L_{II–III}$ isoforms of this channel that might arise from alternative splicing of a different exon. These isoforms of $Ca_V2.1$ differ in their interactions with SNARE proteins and sub-cellular expression patterns [48,64]. Regulated expression of $L_{II–III}$ isoforms could represent a mechanism to limit overall Ca_V2 channel density at the synapse during maturation.

4. Mutually exclusive exons e37a and e37b in the C-terminus of $Ca_V2.2$

4.1. The C-terminus of Ca_V2

The C-termini of Ca_V2 genes coordinate various aspects of calcium channel function, including inactivation, modulation by G-proteins, modulation by calmodulin, and protein-protein interactions that regulate activity and/or target the channel to specific cellular compartments [9,37,47,49,73–76]. Alternative splicing in exon 46 of $Ca_V2.2$ affects the ability of G-proteins to modulate the N-type calcium channel [49] and alters sub-cellular targeting [47,73]. Mutually exclusive e37a and e37b are found in $Ca_V2.1$, $Ca_V2.2$, and $Ca_V2.3$ genes (Fig. 3A). The amino acid compositions of these exons differ among the Ca_V2 genes, and the expression patterns of $Ca_V2.2$ e37a and e37b [7] are distinct from the reported expression patterns of the equivalent exons in $Ca_V2.1$ [9], suggesting that they serve different functions. Notably, we have shown that $Ca_V2.2e[37a]$ is preferentially expressed in capsaicin-responsive dorsal root ganglia neurons, implying a role in nociception [7].

4.2. Exon 37a is expressed preferentially in dorsal root ganglia

$Ca_V2.2$ channels localize to synapses of nociceptive neurons in the dorsal horn laminae I/II [77] and dominate in the control of transmitter release from these presynaptic terminals [78–80]. Mice lacking $Ca_V2.2$ have impaired nociception [81–84], and N-type channel blockers administered intrathecally are powerful analgesics in a variety of animal pain models [85–89]. Some of the analgesic properties of morphine and other opiates are mediated via

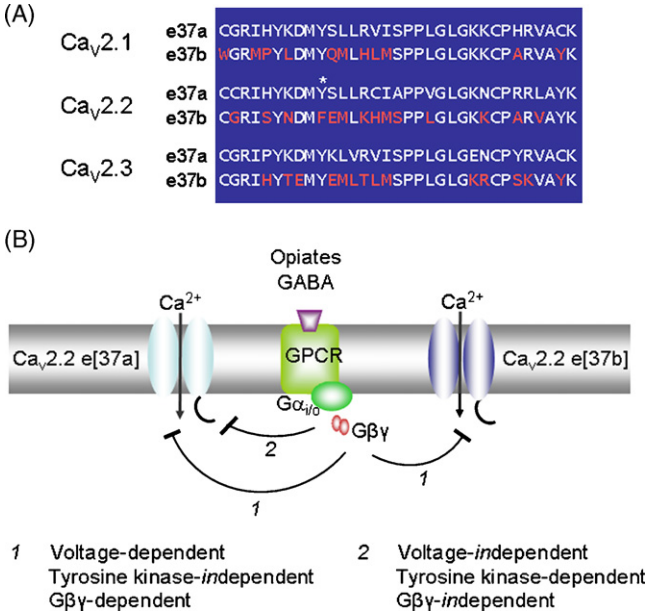

Fig. 3. (A) Amino acid sequence alignments of exons e37a and e37b of human $Ca_V2.1$, $Ca_V2.2$, and $Ca_V2.3$ genes. GenBank accession numbers were: AC011446 ($Ca_V2.1$), AY211499 ($Ca_V2.2e[37a]$), AF055477 ($Ca_V2.2e[37b]$), and AF239258S3 ($Ca_V2.3$). Red font labels amino acids that differ between e37a and e37b. A tyrosine kinase consensus site is indicated by an asterisk at position 1747 in $Ca_V2.2e[37a]$. Tyrosine 1747 is essential for G-protein-mediated voltage-independent inhibition of N-type calcium currents. (B) Schematic showing the two inhibitory pathways that couple to N-type channels in nociceptors. The voltage-dependent inhibitory pathway couples to both $Ca_V2.2e[37a]$ and $Ca_V2.2e[37b]$ channels. Voltage-dependent inhibition utilizes pertussis toxin-sensitive G-proteins and requires Gβγ dimers. Voltage-independent inhibition only couples to $Ca_V2.2e[37a]$ channels. This pathway uses pertussis toxin-sensitive G-proteins, is independent of Gβγ dimers, depends on *src* pp60 tyrosine kinase, and requires a tyrosine at position 1747 in $Ca_V2.2$.

G-protein-dependent inhibition of N-type channels in the dorsal horn. A sub-set of nociceptive neurons preferentially express $Ca_V2.2e[37a]$ mRNAs [7,90]. This novel $Ca_V2.2$ splice isoform supports larger N-type currents in nociceptive neurons, as compared to the more prevalent splice isoforms of $Ca_V2.2$ that contain the alternative exon e37b [7,91]. Larger currents associated with e37a-containing channels impact the amount of calcium entering cells in response to a variety of action potential waveforms [91]. More recently, we showed that e37a increases the sensitivity of the N-type channel to the voltage-independent form of G-protein modulation [92]. Collectively, our findings provide potentially important insights into the cellular mechanisms that control N-type channel activity in nociception and indicate that a sub-set of nociceptive neurons express a form of the N-type channel that is highly sensitive to inhibition by neurotransmitters and hormones.

4.3. G-protein-dependent modulation of N-type channel gating

Having shown preferential expression of e37a in nociceptors of dorsal root ganglia, we considered what features

of N-type channels in these cells are especially prominent. Numerous G-protein-coupled neurotransmitter receptors suppress transmitter release from many neurons by inhibiting N-type calcium channels [30,44,45,93]. G-protein-dependent inhibition can be voltage-dependent and mediated by direct interaction of G-protein βγ subunit dimers with $Ca_V2.2$ subunits [43,44,94]. This inhibition is membrane delimited, slows activation of the N-type current, and can be relieved by a strong depolarizing pre-pulse [44]. Voltage-dependent inhibition is most effective against calcium entry triggered by single action potentials. It is relieved by a train of action potentials [94–96]. Another form of G-protein-dependent inhibition of the N-type current is voltage-independent and this is an especially prominent feature of N-type channels in nociceptors [97]. This form of inhibition is not relieved by strong depolarizing pre-pulses and may involve kinase-dependent phosphorylation by protein kinase C [98] and tyrosine kinases [99]. The contributions of voltage-dependent and voltage-independent inhibition vary with transmitter receptor, cell type, and level of syntaxin 1A [43,44,100–103]. However, the molecular mechanisms that set the level and type of transmitter-mediated inhibition of the N-type channel are unknown. Our recent findings show that the degree and type of G-protein-mediated inhibition of the N-type channel expressed in nociceptors are regulated by alternative splicing of e37a and e37b in the C-terminus of the $Ca_V2.2$ subunit.

4.4. Alternative splicing controls G-protein—dependent inhibition of N-type calcium channels in nociceptors

We were motivated to test the role of e37a in G-protein-mediated inhibition of the N-type channel because we had shown enrichment of e37a in nociceptors, where N-type channels are highly sensitive to inhibition by G-protein-coupled receptors. We found that inclusion of e37a in $Ca_V2.2$ created an inhibitory pathway that was independent of voltage and that substantially increased the sensitivity of $Ca_V2.2$ channels to modulation by opiates and GABA [92]. Voltage-dependent inhibition was present in e37a and e37b-containing N-type channels and was unaffected by alternative splicing at this site in the C-terminus. We also showed that both forms of inhibition, voltage-dependent and voltage-independent, utilize PTX-sensitive G_i and G_o class of G-proteins, but the two pathways diverge downstream of the trimeric G-protein. As described by many investigators, voltage-dependent inhibition requires Gβγ dimer binding to the $Ca_V2.2$ channel and is independent of kinase activation [44,45] (Fig. 3B). By contrast, voltage-independent inhibition proceeds independent of Gβγ and requires pp60c-src tyrosine kinase activation [92,99] (Fig. 3B). Interestingly, there is a tyrosine residue encoded in e37a and absent in e37b that is essential for supporting voltage-independent inhibition of $Ca_V2.2e[37a]$ channels (Fig. 3A). GABA, opiates and probably other transmitters and drugs can utilize this pathway to inhibit $Ca_V2.2$ channels by a mechanism that is independent of stimulus intensity [95,96,104] (Fig. 3B).

5. Conclusions

Our studies of alternative splicing illustrate how investigations of evolutionarily conserved, natural variants of $Ca_V2.2$ can uncover critical domains in N-type channels that regulate their activity. The e18a splice site illustrates that alternative splicing of certain exons in $Ca_V2.2$ is under developmental control and possibly coordinated with splicing of the homologous site in $Ca_V2.3$. Collectively, our studies suggest that N-type channels become less sensitive to inactivation following trains of stimuli during development. Alternative splicing of e37a, on the other hand, is under cell-specific control. Knowledge that e37a-containing $Ca_V2.2$ channels are preferentially expressed in nociceptors provided the functionally relevant context to understand the significance of our findings.

We still know very little of the factors that regulate alternative splicing of voltage-gated calcium channels. Of the proteins that have been identified that regulate neuron-specific alternative splicing, only the Nova2 RNA binding protein has been linked to $Ca_V2.2$ [105]. As factors that regulate expression of alternatively spliced exons in $Ca_V2.2$ channels are identified, we should begin to gain a better understanding of the mechanisms that exist to regulate calcium channel function and to control synaptic efficacy.

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