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Cell-specific alternative splicing in N-type calcium channels controls their sensitivity to G protein coupled receptors

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Cell-specific alternative pre-mRNA splicing is thought to optimize protein function for specialized cellular tasks, but direct evidence for this is lacking. With this in mind, we devised a mouse model to determine the functional importance of the mutually exclusive splice site e37a/e37b in the *CACNA1B* gene (Cav2.2/N-type calcium channel). We eliminated e37a from the mouse *CACNA1B* gene and replaced it with a second 37b-encoding exon. Similarly, in a second mouse line, we eliminated e37b and replaced it with a second 37a-encoding exon. Using these mice we showed that e37a regulates mu-opioid Gi/o-protein receptor-mediated inhibition of N-type calcium channels in nociceptors of dorsal root ganglia. We also showed that e37a enhances spinal level analgesia by morphine *in vivo* without affecting basal transmission of noxious thermal stimuli.

In a different set of experiments we showed that Fox-2, a neuronal specific splice factor controls selection of e18a, another alternatively spliced exon in the *CACNA1B* gene. Fox-2 represses e18a inclusion and we show that siRNA against Fox-2 shifts the balance of splicing toward e18a-containing Cav2.2 mRNAs in neurons. Exon 18a supports increased N-type current density and reduced sensitivity to Gs-protein coupled receptors when studied in a recombinant expression system. We are now using Fox-2 siRNAs to show how e18a in controls N-type channel activity in sympathetic neurons. Collectively our studies provide direct evidence that cell-specific alternative splicing controls the way Gi/o and Gs proteins signal to neuronal N-type calcium channels.

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