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### Alternative Splicing Matters: N-Type Calcium Channels in Nociceptors

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## Addendum

# Alternative Splicing Matters

## N-Type Calcium Channels in Nociceptors

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Addendum to:

*Alternative Splicing Controls G Protein-Dependent Inhibition of N-Type Calcium Channels in Nociceptors*

Raingo J, Castiglioni AJ and Lipscombe D

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### ABSTRACT

How many different calcium channels does it take to make a nervous system? The answer: more than any of us predicted. In 1975 Hagiwara and colleagues published the first evidence that functionally different calcium channels are expressed in cells.<sup>1</sup> By 1999, the calcium channel family could boast ten members, each member defined by a unique set of attributes to support their cellular functions and by unique amino acid sequences.<sup>2</sup> Although nine of these genes are expressed in the nervous system, that number still seemed insufficient to support the wide spectrum of neuronal functions controlled by voltage-gated calcium channels. This discrepancy is probably explained by alternative pre-messenger RNA splicing which substantially expands the number of protein activities available from a limited number of genes. Like many other ion channel genes, each  $Ca_v\alpha_1$  gene has the capacity to generate perhaps thousands of unique splice isoforms with unique functional properties.<sup>3</sup> The high level of conservation among alternatively spliced exons in  $Ca_v2.2$  genes of different species and in some cases closely related genes implies biological importance. A number of  $Ca_v\alpha_1$  isoforms have been identified from neural tissue but until recently we lacked direct evidence linking a specific splice site in a calcium channel gene to a specific function in an identified neuron population.

Our recent studies show that alternative pre-mRNA splicing of a pair of 32 amino acid encoding exons in the C-terminus of  $Ca_v2.2$ , e37a and e37b, underlie the expression of two mutually exclusive N-type channel isoforms. The inclusion of e37a creates a module that couples the N-type channel to a powerful form of G protein-dependent inhibition.<sup>4</sup> The inhibitory pathway that works through e37a is voltage-independent, requires  $G_{i/o}$  and tyrosine kinase activation, and is used by mu opioid and GABA<sub>B</sub> receptors to downregulate N-type channel activity. Combined with our previous studies that show enrichment of e37a in nociceptors,<sup>5</sup> our data suggest a molecular basis for the high susceptibility of N-type currents in sensory neurons to voltage-independent inhibition following G protein activation.<sup>6</sup>

### OPENING THE GATE TO G PROTEIN-MEDIATED INHIBITION

N-type calcium channels in most neurons are susceptible to inhibition by G protein activation.<sup>7,8</sup> This ubiquitous form of inhibition is voltage-dependent and mediated by G protein  $G\beta\gamma$  dimers. Functionally, voltage-dependent inhibition is analogous to a low pass filter. It inhibits brief stimuli but, because it is relieved by depolarization, voltage-dependent inhibition of N-type currents permits the passage of intense, high frequency stimuli.<sup>7</sup> Some N-type currents are also susceptible to voltage-independent inhibition.<sup>7,8</sup> A  $G_{i/o}$ -dependent form of voltage-independent inhibition of N-type currents is well described in chick sensory neurons<sup>9</sup> whereas  $G_i$  couples voltage-independent inhibition to N-type currents in sympathetic neurons.<sup>8,10</sup> Voltage-dependent inhibition and voltage-independent inhibition are additive; they work in concert to dampen N-type channel activity over a range of stimulus intensities and frequencies. Our studies explain why N-type currents in sensory neurons are highly susceptible to voltage-independent inhibition that uses  $G_{i/o}$ . We show that voltage-independent inhibition mediated by  $G_{i/o}$  can only gain access to the N-type channel if the  $Ca_v2.2$  subunit contains e37a.<sup>4</sup>

### WHAT TOOK US SO LONG?

We initially identified e37a in  $Ca_v2.2$  in 2004 but did not immediately recognize its importance for G protein-dependent, voltage-independent inhibition of the N-type channel in sensory neurons. In retrospect, the answer seems obvious given the elegant studies in the literature from several groups who defined the signaling pathways underlying

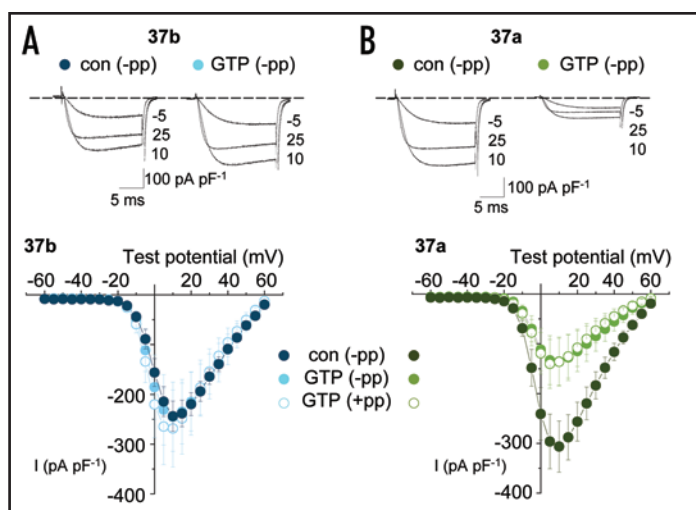


Figure 1. Internal GTP alone triggers voltage-independent inhibition of  $\text{Ca}_v2.2[\text{e}37\text{a}]$  N-type channels. Calcium currents recorded from cells expressing  $\text{Ca}_v2.2[\text{e}37\text{b}]$  (A) and  $\text{Ca}_v2.2[\text{e}37\text{a}]$  (B) channels under control conditions (con) and in the presence of 0.4 mM GTP (+pp, -pp). Currents were evoked by test pulses alone (con, -pp) or preceded by a prepulse to +80 mV (+pp). Exemplar  $\text{Ca}_v2.2[\text{e}37\text{b}]$  (A) and  $\text{Ca}_v2.2[\text{e}37\text{a}]$  (B) currents were evoked by test potentials of -5 mV, +10 mV and +25 mV. In the presence of GTP, prepulses to +80 mV did not recover any of inhibited  $\text{Ca}_v2.2[\text{e}37\text{a}]$  current (B).  $\text{Ca}_v2.2[\text{e}37\text{b}]$  currents (A) were unaffected by GTP. N values are  $n = 4$  (con) and  $n = 6$  (GTP; -pp, +pp) (A) and  $n = 5$  (con) and  $n = 4$  (GTP; -pp, +pp) (B).

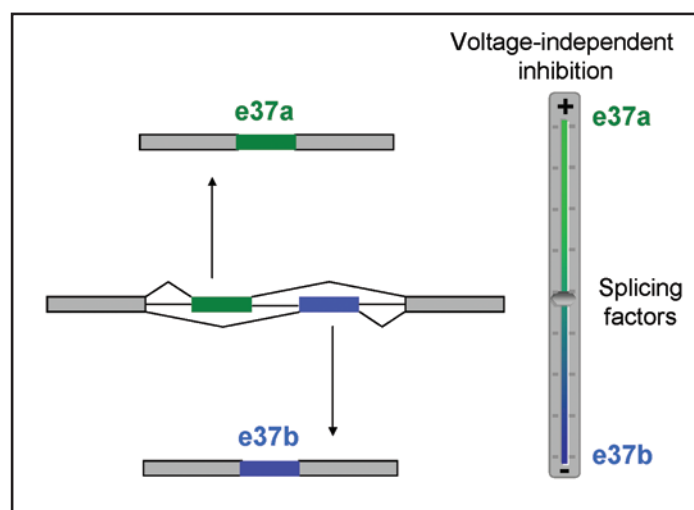


Figure 2. Cells may control the sensitivity of their N-type channels to G protein-mediated voltage-independent inhibition by adjusting the ratio of e37a to e37b-containing  $\text{Ca}_v2.2$  mRNAs.

of native N-type currents in chick sensory neurons is triggered by low concentrations of transmitter (GABA and norepinephrine), whereas higher concentrations of transmitter are needed to trigger voltage-dependent inhibition (which they called kinetic slowing).<sup>6</sup>

## HOW IMPORTANT IS e37a TO NOCICEPTION?

N-type channels in nociceptors are implicated in chronic pain. Inhibitors of N-type currents are effective in treating neuropathic pain and several drugs and endogenous transmitters with analgesic actions in part act through inhibition of presynaptic N-type channels of sensory neurons in the dorsal horn of the spinal cord. Do e37a and e37b-containing N-type channels contribute similarly to nociception? A recent study from the lab of Gerald Zamponi that includes contributions from Dickenson, Vergnolle and our labs, conclude: no.<sup>13</sup> Using splice isoform-specific small interfering RNA, Zamponi and collaborators showed that only e37a-containing N-type channels appear to mediate transmission of thermal nociception both in basal conditions (undamaged tissues) and in inflammatory or neuropathic pain models.<sup>13</sup> These studies suggest that selective inhibitors of e37a-containing N-type channels could be particularly effective in the treatment of certain forms of pain. More generally, they also suggest that e37a splice isoforms are present at presynaptic nociceptive nerve terminals to regulate synaptic transmission.

## CAN SINGLE CELLS CONTROL THEIR e37a:e37b RATIO?

In theory, nociceptors could increase the sensitivity of their N-type channels to neurotransmitters and drugs by shifting from e37b-containing to e37a-containing  $\text{Ca}_v2.2$  mRNAs (Fig. 2). This could be achieved by controlling inclusion of e37a at the level of pre-messenger RNA. An increase in  $\text{Ca}_v2.2[\text{e}37\text{a}]$  mRNA levels in nociceptors should increase N-type channel sensitivity to opioids and GABA, particularly during periods of intense sensory input. Conversely, a decrease in  $\text{Ca}_v2.2[\text{e}37\text{a}]$  N-type channels, which we showed recently occurs in dorsal root ganglia following peripheral nerve injury,<sup>13</sup> could decrease N-type channel sensitivity to drugs

voltage-dependent and voltage-independent inhibition of native N-type currents (refs. 7, 8 and 11). However, our early experiments threw us off track, initially we did not find differences in the responsiveness of  $\text{Ca}_v2.2[\text{e}37\text{a}]$  and  $\text{Ca}_v2.2[\text{e}37\text{b}]$  N-type currents to  $\text{GTP}\gamma\text{S}$ ; both appeared to exhibit only voltage-dependent inhibition. Later we discovered that the voltage-independent inhibitory pathway targeting the e37a isoform of  $\text{Ca}_v2.2$  appeared activated in our control recordings. The culprit, internal GTP, which appears to activate the voltage-independent inhibitory pathway in cells expressing  $\text{Ca}_v2.2[\text{e}37\text{a}]$  channels (Fig. 1). In the absence of internal GTP,  $\text{Ca}_v2.2[\text{e}37\text{a}]$  currents were larger than  $\text{Ca}_v2.2[\text{e}37\text{b}]$  currents (see Fig. 2 in ref. 12 and Fig. 1 in ref. 4) and when stimulated by  $\text{GTP}\gamma\text{S}$ ,  $\text{Ca}_v2.2[\text{e}37\text{a}]$  currents exhibited voltage-dependent and voltage-independent inhibition (see Fig. 1 in ref. 4). By contrast, internal GTP had no effect on  $\text{Ca}_v2.2[\text{e}37\text{b}]$  currents, suggesting that GTP activates only the voltage-independent, not the voltage-dependent pathway to the N-type channel (Fig. 1). Notably, we also showed that both inhibitory pathways to e37a-containing N-type channels were intact and could be activated by receptor stimulation using the perforated patch recording method (see Figs. 3 and 4 in ref. 4).

GTP (0.2–0.3 mM) is commonly included in internal recording solutions to ensure sufficient levels for G protein-dependent signaling to ion channels. Our data suggest that GTP alone may be sufficient to activate certain G protein-dependent signaling pathways. Our findings also suggest that the voltage-independent pathway that requires e37a to inhibit the N-type channel may be more sensitive to GTP and perhaps to G protein stimulation. Our data are consistent with studies from the Dunlap lab. They showed that voltage-independent inhibition (which they called steady-state inhibition)

and neurotransmitters. Currently, the cell-specific splicing factors that promote e37a inclusion in  $Ca_v2.2$  mRNA are unknown. Identifying these factors and determining how they are regulated promise to be exciting areas of future research.

## WHAT DOES e37a DO IN OTHER $Ca_v2$ GENES?

The e37a/e37b splice junction is present in all three  $Ca_v2$  genes although their sequences differ among the three genes.<sup>14,15</sup> The functional importance of splicing in  $Ca_v2.3$  is not known but there is evidence that in  $Ca_v2.1$  this site works in combination with a second site of alternative splicing in the C-terminus (e47) to regulate calcium dependent facilitation in P-type channels.<sup>16</sup> It would be interesting to test the possibility that alternative splicing of equivalent exons in  $Ca_v2.1$  and  $Ca_v2.3$  also control G protein signaling to P-type and R-type channels, respectively. Our own studies of e37a in  $Ca_v2.2$  suggest this exon is multifunctional. It regulates G protein signaling to the N-type channel as well as current density.<sup>12</sup> Overall, e37a increases the dynamic range of the N-type current by increasing the maximum current relative to e37b channels while also creating an additional inhibitory pathway for use by neurotransmitter and drugs to dampen channel activity. It is therefore possible that this domain in  $Ca_v2.1$  and  $Ca_v2.3$  subunits have similar multifunctional qualities.

### References

1. Hagiwara S, Ozawa S, Sand O. Voltage clamp analysis of two inward current mechanisms in the egg cell membrane of a starfish. *J Gen Physiol* 1975; 65:617-44.
2. Perez-Reyes E. Molecular physiology of low-voltage-activated t-type calcium channels. *Physiol Rev* 2003; 83:117-61.
3. Lipscombe D. Neuronal proteins custom designed by alternative splicing. *Curr Opin Neurobiol* 2005; 15:358-63.
4. Raingo J, Castiglioni AJ, Lipscombe D. Alternative splicing controls G protein-dependent inhibition of N-type calcium channels in nociceptors. *Nat Neurosci* 2007; 10:285-92.
5. Bell TJ, Thaler C, Castiglioni AJ, Helton TD, Lipscombe D. Cell-specific alternative splicing increases calcium channel current density in the pain pathway. *Neuron* 2004; 41:127-38.
6. Diverse-Pierluissi M, Goldsmith PK, Dunlap K. Transmitter-mediated inhibition of N-type calcium channels in sensory neurons involves multiple GTP-binding proteins and subunits. *Neuron* 1995; 14:191-200.
7. Ikeda SR, Dunlap K. Voltage-dependent modulation of N-type calcium channels: Role of G-protein subunits. *Adv Second Messenger Phosphoprotein Res* 1999; 33:131-51.
8. Hille B, Beech DJ, Bernheim L, Mathie A, Shapiro MS, Wollmuth LP. Multiple G-protein-coupled pathways inhibit N-type Ca channels of neurons. *Life Sci* 1995; 56:989-92.
9. Diverse-Pierluissi M, Remmers AE, Neubig RR, Dunlap K. Novel form of crosstalk between G protein and tyrosine kinase pathways. *Proc Natl Acad Sci USA* 1997; 94:5417-21.
10. Kammermeier PJ, Ruiz-Velasco V, Ikeda SR. A voltage-independent calcium current inhibitory pathway activated by muscarinic agonists in rat sympathetic neurons requires both  $G\alpha_q/11$  and  $G\beta\gamma$ . *J Neurosci* 2000; 20:5623-9.
11. Ikeda SR, Dunlap K. Calcium channels diversify their signaling portfolio. *Nat Neurosci* 2007; 10:269-71.
12. Castiglioni AJ, Raingo J, Lipscombe D. Alternative splicing in the C-terminus of  $Ca_v2.2$  controls expression and gating of N-type calcium channels. *J Physiol* 2006; 576:119-34.
13. Altier C, Dale CS, Kisilevsky AE, Chapman K, Castiglioni AJ, Matthews EA, Evans RM, Dickenson AH, Lipscombe D, Vergnolle N, Zamponi GW. Differential role of N-type calcium channel splice isoforms in pain. *J Neurosci* 2007; 27:6363-73.
14. Gray AC, Raingo J, Lipscombe D. Neuronal calcium channels: Splicing for optimal performance. *Cell Calcium* 2007; 42:409-17.
15. Bourinet E, Soong TW, Sutton K, Slaymaker S, Mathews E, Monteil A, Zamponi GW, Nargeot J, Snutch TP. Splicing of  $\alpha1A$  subunit gene generates phenotypic variants of P- and Q-type calcium channels. *Nat Neurosci* 1999; 2:407-15.
16. Chaudhuri D, Chang SY, DeMaria CD, Alvania RS, Soong TW, Yue DT. Alternative splicing as a molecular switch for  $Ca^{2+}$ /calmodulin-dependent facilitation of P/Q-type  $Ca^{2+}$  channels. *J Neurosci* 2004; 24:6334-42.