Functional Impact of Alternative Splicing of Human T-Type Cav3.3 Calcium Channels

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Murbartia, Janet, Juan Manuel Arias, and Edward Perez-Reyes. Functional impact of alternative splicing of human T-type Cav3.3 calcium channels. J Neurophysiol 92: 3399–3407, 2004. First published July 14, 2004; 10.1152/jn.00498.2004. Low-voltage-activated T-type (Ca,3) Ca2+ channels produce low-threshold spikes that trigger burst firing in many neurons. The CACNA1I gene encodes the Ca,3.3 isoform, which activates and inactivates much more slowly than the other Ca,3 channels. These distinctive kinetic features, along with its brain-region-specific expression, suggest that Ca,3.3 channels endow neurons with the ability to generate long-lasting bursts of firing. The human CACNA1I gene contains two regions of alternative splicing: variable inclusion of exon 9 and an alternative acceptor site within exon 33, which leads to deletion of 13 amino acids (Δ33). The goal of this study is to determine the functional consequences of these variations in the full-length channel. The cDNA encoding these regions were cloned using RT-PCR from human brain, and currents were recorded by whole cell patch clamp. Introduction of the Δ33 deletion slowed the rate of channel opening. Addition of exon 9 had little effect on kinetics, whereas its addition to Δ33 channels unexpectedly slowed both activation and inactivation kinetics. Modeling of neuronal firing showed that exon 9 or Δ33 alone reduced burst firing, whereas the combination enhanced firing. The major conclusions of this study are that the intracellular regions after repeats I and IV play a role in channel gating, that their effects are interdependent, suggesting a direct interaction, and that splice variation of Ca,3.3 channels provides a mechanism for fine-tuning the latency and duration of low-threshold spikes.

INTRODUCTION

The intrinsic firing properties of neurons are regulated by the types of ion channel genes that they express (Llinás 1988) and by alternative splicing of those genes (Bourinet et al. 1999). T-type Ca2+ channels open after small depolarizations of the plasma membrane and hence mediate low-threshold Ca2+ spikes. These spikes in turn mediate the opening of voltage-gated Na+ channels, and a second set of high-voltage-activated Ca2+ channels (reviewed in Perez-Reyes 2003). Therefore neuronal firing can be fine-tuned by the kinetics and voltage sensitivity of T-type Ca2+ channels.

From the cloning and expression of their cDNAs, it is now known that there are three T-type channel genes. The channels encoded on these genes (Ca,3.1, Ca,3.2, and Ca,3.3) have distinct kinetic features, suggesting that they play distinct roles in neuronal firing (Kozlov et al. 1999). Specifically, the slow activation and inactivation of Ca,3.3 channels suggest they play a role in sustained firing, whereas the fast kinetics of Ca,3.1 and Ca,3.2 would lead to firing of short bursts (Chemin et al. 2002).

Alternative splicing of Ca2+ channel genes is known to play an important role in determining their channels’ biophysical properties, their pharmacology, and their ability to be regulated by G proteins. Notable examples include the splicing of CACNA1C, which modulates its sensitivity to antihypertensive drugs (Welling et al. 1997), and splicing of CACNA1A, which modulates its sensitivity to spider toxins used to differentiate P- from Q-type channels (Bourinet et al. 1999). Completion of the human genome sequence facilitated study of alternative splicing by allowing identification of intron-exon boundaries and the design of PCR primers to span the splice junctions. Using this approach, Mittman and coworkers identified two sites of alternative splicing in the human CACNA1I gene (Mittman et al. 1999). One site is the variable inclusion of exon 9 (abbreviated +9 or Δ9), which adds 35 amino acids, whereas the second site is the use of an alternate acceptor in exon 33 (abbreviated +33 or Δ33), which leads to variable inclusion of 13 amino acids (Mittman et al. 1999). Although the effect of exon 33 splicing on human Ca,3.3 channel activity was studied by Chemin and coworkers, these studies used a truncated channel that was missing exon 37 (see (Gomora et al. 2002). Exon 37 encodes half of the carboxy terminal sequence that follows the last transmembrane segment (IVS6) and has been shown to play a role in channel regulation (Gomora et al. 2002). The rat Ca,3.3 gene is also alternatively spliced at the homologous region as exon 33, and the electrophysiological consequences of this splicing are modulated by the distal carboxy terminus (Murbartían et al. 2002). This splicing was found to occur in a brain-region-specific manner, suggesting that alternative splicing may provide a mechanism for fine tuning neuronal firing. Therefore the goals of this study were to investigate the electrophysiological consequences of exon 9 and 33 splicing in a full-length human Ca,3.3 channel.

Furthermore, the NEURON model was used to predict the functional impact that this splicing might have on neuronal firing (Hines and Carnevale 2001). This model has been used extensively to validate the role of T-type channels in neuronal excitability, in particular in neurons of the thalamocortical loop (Destexhe et al. 1994, 1996a, 1998a; Lytton et al. 1997). Notably, models have been developed for neurons of the reticular nucleus of the thalamus, where Ca,3.3 mRNA is highly expressed. Burst firing of these neurons is mediated by a slowly inactivating T current (Huguenard and Prince 1992) and plays an important role in generating slow-wave (2–4 Hz)
discharges observed in absence epilepsy patients and in other thalamocortical dysrhythmias (Jeanmonod et al. 1996).

METHODS

Molecular cloning

cDNA was synthesized from 100 ng fetal brain poly(A) RNA (BD Biosciences Clontech, Palo Alto, CA) using 5 μM random nonamers, 0.5 mM of dNTP, 20 U SUPERase· In (Ambion, Austin, TX), and 100 U M-MLV reverse transcriptase (Ambion). Amplification reactions (25 μl volume) were performed in a Mastercycler gradient (Eppendorf, Westbury, NY) and contained 2.5 μl cDNA, 0.4 μM each primer, 0.2 mM dNTP, and 0.5 U Vent DNA polymerase (New England Biolabs, Beverly MA). After a 150-s denaturation step, reactions were cycled 35 times using 25 s for denaturation (94°C), annealing (62°C), and extension (72°C). PCR products were then treated with 1 U Taq DNA polymerase and 0.2 mM dATP and cloned into pCRII-TOPO kit (Invitrogen, Carlsbad, CA). Colonies were screened by PCR amplification with the respective PCR primers. Positive clones were further identified by their restriction enzyme map, and sequencing. Synthetic oligonucleotides used for DNA amplifications were obtained from Operon (Alameda, CA). Splicing of exon 9 was assessed using primers u5 and a22, which are located in exons 8 and 10 (Fig. 1 A). Splicing of exon 33 was assessed using PCR primers n43 and r7, which are located in exons 32 and 34 (Fig. 1B).

The starting material for construction of full-length cDNAs was the human Cav3.3 clone LT9 (GenBank Accession No. AF393329), the cloning of which was described previously (Gomora et al. 2002). To facilitate addition of the exon 9 sequence, a silent mutation was introduced that would create a BspEI restriction enzyme site. This was accomplished in two PCR reactions: the first reaction used primers u5 and r22 and plasmid derived from the PCR product shown in Fig. 1, and the second reaction used primers u10 and r21. The full-length cDNA was generated by ligating the following fragments: CiaI (polylinker)/BsaI (1220) from LT9, the BsaIBspEI fragment for PCR1, the BspEI/AvaII fragment from PCR2, and the AvaII (2683)/EcoRI (polylinker) fragment from LT9. The A33 variation was introduced by single overlap extension (SOE) method (Horton et al. 1993) using 0.5 U Vent DNA polymerase. The full-length cDNA was generated by ligating the following fragments: EcoRI (polylinker)/HindIII (4755) from LT9, the HindIII/BamHI fragment of the SOE fragment, and the BamHI (5662)/XbaI (polylinker) fragment from LT9.

Transfections

Two hundred ninety three cells (human embryonic kidney, No. CRL-1573, American Type Culture Collection, Manassas, VA) were transiently cotransfected with plasmid DNAs encoding each Ca3,3 variant and green fluorescent protein (GFP; pGreen Lantern, Invitrogen), at a molar ratio of 5:1, by the calcium phosphate method (CalPhos Maximizer Transfection Kit, Clontech). After ~24 h, GFP positive cells were selected for electrophysiological recordings. The results were obtained from 12 transfections, and each construct was tested in ≥4 transfections.

Electrophysiology

Electrophysiological experiments were carried out using the whole cell configuration of the patch-clamp technique. Recordings were obtained using an Axopatch 200B amplifier, Digidata 1322 A/D converter, and pCLAMP 8.0 software (Axon Instruments, Union City, CA). Data were filtered at 2 kHz and digitized at 5 kHz. Tail currents were filtered at 10 kHz and digitized at 50 kHz. Whole cell Ca2+ currents were recorded using the following external solution (in mM): 5 CaCl2, 155 tetraethyl ammonium (TEA) chloride, and 10 HEPES, pH adjusted to 7.4 with TEA-OH. The internal pipette solution contained 130 mM K+ as choline, 40 mM Na+, 1 mM Mg2+, and 100 mM EGTA, pH adjusted to 7.4 with choline-OH. Data were analyzed using Igor Pro (Wavemetrics, Lake Oswego, OR) and Clampfit (Axon Instruments, Union City, CA).

FIG. 1. PCR amplification of exon 9 and exon 33 splice variants. A: the location and amino acid sequence encoded by exon 9 is shown. The channel variant lacking exon 9 is given (-) and labeled Δ9. The alignment of human Cav3,3 sequences begins in the sixth transmembrane segment of repeat 1 (IS6), and the numbering is that of the human Cav3.3a repeat (IVS6), and the numbering is that of the line. B: location and sequence of exon 33 variants. The alignment begins at the end of the sixth transmembrane segment of the 4th repeat (IVS6), and the numbering is that of human Cav3.3a (Δ9 + 33). C: photograph of an ethidium-bromide-stained agarose gel showing the PCR products. Lane 1 contained the 100-bp size markers. Lane 2 shows the PCR product amplified by primers u5 and a22. Transcripts containing exon 9 were specifically amplified using u5 in combination with the primer r22. Lane 4 shows the PCR product amplified by n43 and r7 primers, which brackets exon 33. Lanes 5 and 6 show the negative control reactions that lack either reverse transcriptase in the cDNA synthesis step (no RT) or that lack cDNA template in the PCR reaction. The expected size of the products is indicated on the right.
contained the following (in mM): 125 CsCl, 10 EGTA, 2 CaCl2, 1 MgCl2, 4 Mg-ATP, 0.3 Na3GTP, and 10 HEPES, pH adjusted to 7.2 with CsOH. Pipettes were made from TW-150-3 capillary tubing (World Precision Instruments, Sarasota, FL). Under these solution conditions, the pipette resistance was typically 2–3 MΩ. Access resistance in the whole cell configuration averaged 5.6 ± 0.2 MΩ (n = 40) and was compensated between protocols to ≥70%. Cell capacitance averaged 10.9 ± 0.5 pF (n = 40). All experiments were performed at room temperature (−22°C).

Data analysis

PCR product formation was quantitated using densitometric analysis as described previously (Murbartia et al. 2002). Peak currents and exponential fits to currents were determined using Clampfit 8.0 software (Axon Instruments). Leak subtraction was performed off-line using the passive resistance algorithm in Clampfit. Fits to average data and statistical tests were performed using Prism software (Graphpad, San Diego, CA). Statistical tests included ANOVA, unpaired two-tailed Student’s t-test for comparing two data sets, and the F test for comparing two models such as single- or double-exponential fits. Statistically significant differences are noted with a single asterisk if P < 0.05 and with a double asterisk if P < 0.01. Average data are presented as means ± SE.

Modeling

The NEURON model uses mathematical descriptors of ionic conductances to calculate whether a channel is open as a function of voltage, and does this by applying Hodgkin-Huxley-type equations. The voltage dependence of activation (τa) and inactivation (τh) was determined as in the preceding text. The data were corrected for a junction potential (−9.4 mV; Figs. 1–4 show uncorrected data) and surface charge screening (−4 mV). The data were collected at 24°C and the simulations were at 36°C. The model accounts for this difference using Q10 values determined previously (Coulter et al. 1989). The maximum permeability of T currents was left as in the original models. The experimentally determined values of the recombinant channels were then used to replace all other parameters for the T-channel in the models of thalamic reticular neurons (Destexhe et al. 1996b). The current-clamp simulations used the three-compartment model with the current injected into the dendritic compartment and recorded from the virtual soma.

RESULTS

Alternative splicing of exons 9 and 33 in whole fetal brain RNA were individually analyzed using RT-PCR with flanking primers (Fig. 1). The abundance of each splice variant can be estimated from ethidium-bromide-stained agarose gels because product formation was in the linear range (data not shown), and the PCR primers are the same for each exon. Densitometric analysis indicated that the predominant transcripts are those that skip exon 9 (Δ9) and contain the full exon 33 sequence (abbreviated Δ9 + 33). The ratio of Δ9 to +9 transcripts was ~10:1. Transcripts containing exon 9 were readily detected using a reverse primer based on the exon 9 DNA sequence. PCR products containing the complete exon 33 sequence were more abundant than those resulting from the internal acceptor site (Δ33). The ratio of +33 to Δ33 was ~5:1. The PCR products were subcloned, and the plasmid DNA from 48 colonies was analyzed by restriction mapping and sequencing of representative clones. Of these, 41 were +33 and 7 were Δ33. These results confirm the identity of the PCR products and their relative abundance.

To assess the electrophysiological consequences of alternative splicing on Ca3,3 channel activity, full-length cDNAs were constructed, transfected into HEK-293 cells, then studied using patch-clamp electrophysiology. The variants were introduced into the full-length Ca3,3 clone LT9, which lacks exon 9, contains the full exon 33 (Δ9 + 33), and includes exon 37 (Gomora et al. 2002). All four splice constructs led to the induction of robust low-voltage-activated currents with peak current densities >100 pA/pF. The current-voltage (I-V) relationships were identical for all variants (Table 1). Visual inspection of normalized current traces (Fig. 2A) revealed that truncation of exon 33 (Δ33) led to channels that activated
Voltage dependence of current activation ($m_\alpha$)

<table>
<thead>
<tr>
<th>Voltage (V)</th>
<th>$\Delta m_\alpha$</th>
<th>$\Delta m_\alpha$</th>
<th>$m_\alpha$</th>
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<tbody>
<tr>
<td>$V_{1/2}$, mV</td>
<td>-38.5 ± 1.0 (12)</td>
<td>-37.2 ± 0.8 (11)</td>
<td>-38.7 ± 1.0 (9)</td>
<td>-38.1 ± 1.4 (7)</td>
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<tr>
<td>Slope ($k$), mV</td>
<td>7.3 ± 0.2 (12)</td>
<td>7.2 ± 0.3 (11)</td>
<td>6.8 ± 0.3 (9)</td>
<td>7.6 ± 0.1 (7)</td>
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Voltage dependence of current inactivation ($h_\alpha$)

<table>
<thead>
<tr>
<th>Voltage (V)</th>
<th>$\Delta h_\alpha$</th>
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<th>$h_\alpha$</th>
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<tbody>
<tr>
<td>$V_{1/2}$, mV</td>
<td>-72.6 ± 0.6 (11)</td>
<td>-71.0 ± 0.7 (11)</td>
<td>-73.1 ± 1.2 (8)</td>
</tr>
<tr>
<td>Slope ($k$), mV</td>
<td>-5.4 ± 0.2 (11)</td>
<td>-5.4 ± 0.2 (8)</td>
<td>-4.8 ± 0.2 (8)*</td>
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Current kinetics at -40 mV

<table>
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<tbody>
<tr>
<td>Activation ($\tau_\alpha$)</td>
<td>31 ± 4 (12)</td>
<td>45 ± 3 (11)**</td>
<td>36 ± 4 (9)</td>
<td>27 ± 3 (10)</td>
</tr>
<tr>
<td>Inactivation rate ($\tau_\alpha$)</td>
<td>105 ± 5 (12)</td>
<td>111 ± 9 (11)</td>
<td>91 ± 2 (9)*</td>
<td>94 ± 9 (10)</td>
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Current kinetics at 0 mV

<table>
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</tr>
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<tbody>
<tr>
<td>Activation ($\tau_\alpha$)</td>
<td>5.1 ± 0.2 (12)</td>
<td>6.2 ± 0.3 (11)</td>
<td>5.0 ± 0.2 (9)</td>
<td>5.1 ± 0.5 (10)</td>
</tr>
<tr>
<td>Inactivation rate ($\tau_\alpha$)</td>
<td>83 ± 3 (12)</td>
<td>90 ± 4 (11)</td>
<td>79 ± 4 (9)</td>
<td>66 ± 5 (10)**</td>
</tr>
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Recovery from inactivation at -60 mV

| Recovery | 622 ± 45 (4) | 530 ± 71 (4) | 457 ± 22 (3)* | 529 ± 44 (5) |

Table 1. Summary of electrophysiological properties of Ca$^{2+}$ currents from recombinant human Ca$_{3.3}$ splice variants

Statistically significant differences between the splice variants and the Ca$_{3.3}$a ($\Delta m_\alpha + 33$) isoform are noted if the P value was <0.05 (*) or <0.01 (**). Data are expressed as mean values ± SE with the number of cells (n) shown in parentheses.
DISCUSSION

Alternative splicing of high-voltage-activated (HVA) calcium channel subunits can have profound effects on their biophysical and pharmacological properties. In contrast, much less is known about the effects of splicing in low-voltage-activated (LVA) T-type channels. Sequencing of the human genome facilitated the cloning of the CACNA1I subunits of T-type channels and their splice variants (Perez-Reyes 2003). The CACNA1I gene encodes Cav3.3 channels, and the gene is alternatively spliced at exons 9 and 33 (Mittman et al. 1999).

FIG. 2. Effect of exon 33 splicing on Cav3.3 kinetics. A: representative current traces were normalized to the peak current and superimposed. The voltage protocol is shown above. - - -, the fit to the data using a double-exponential equation. B: average $\tau_m$ for Cav3.3 channel variants that lacked exon 9 but either contained the full exon 33 sequence ($\Delta9 + 33$; ○, ●) or the partial deletion ($\Delta9 \Delta33$; ○, ●), or the values obtained from exponential fits to the currents recorded during $I-V$ protocols, as shown in A ($n = 11–12$). ● and ●, the slow activation $\tau$ obtained from double-exponential fits to tail currents (detailed in METHODS; $n = 7$). Statistically significant differences are noted as follows: *$P < 0.05$ or **$P < 0.01$. Error bars are smaller than most symbols. C: average $\tau_h$ for the same 2 Cav3.3 variants (same symbols as in A). The data at $-100$ and $-90$ mV were obtained from protocols measuring the rate of recovery from inactivation ($n = 4–7$). The data at $-70$ and $-60$ mV represent the rate at which inactivation develops at these potentials (weighted $\tau_i$, $n = 3–4$). The data at more depolarized potentials represent $\tau_i$ measured during the $I-V$ protocol as shown in A ($n = 11–12$). Curves in B and C represent fits to the data using the equation described in METHODS.

FIG. 3. Effect of exon 9 on Cav3.3 channels containing the full exon 33 sequence. A: representative current traces showing the recovery of currents after 10 s of inactivation at $-60$ mV. Six sweeps recorded from cells transfected with either $\Delta9 + 33$ (—) or $+9 + 33$ (-----) channels were superimposed. B: average recovery was plotted as a function of repolarization time at $-90$ mV (means ± SE, $n = 3–4$ cells). The data were normalized to the recovery observed after 3 s at $-90$ mV. Time points that were statistically different are noted with * ($P < 0.05$). —, fit to the average data with one exponential. C: the value for $\tau_h$ obtained by averaging the fits to each cell. Data were obtained from protocols to measure recovery, development of inactivation or $I-V$ protocols as described in the Fig. 2 legend.

**SPLICE VARIATION OF A T-TYPE CHANNEL**

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splicing events would lead to changes in the sequence of cytoplasmic loops immediately after the last transmembrane segment of repeats I and IV. The analogous regions in HVA channels are very important for channel regulation by Ca\(^{2+}\) channel β subunits, G protein βγ dimers, and calmodulin. Therefore we hypothesized that they might be important in Ca\(_{\text{LVA}}\) channel function as well. This hypothesis was supported by previous studies of exon 33 splicing using rat and human Ca\(_{\text{LVA}}\) channels (Chemin et al. 2001; Murbartía et al. 2002). When expressed in 293 cells, the human Ca\(_{\text{LVA}}\) exon 33 variants were found to deactivate at different rates but activate and inactivate at essentially the same rate (Chemin et al. 2001). In contrast, the present study found that these variants differ in activation kinetics with no significant difference in deactivation and inactivation kinetics. These disparate results might be explained by the previous study using a channel that was missing the last 214 amino acids of the carboxy terminus encoded by exon 37 (Gomora et al. 2002). Support for this notion comes from studies on rat Ca\(_{\text{LVA}}\) splice variants, where the effects of splicing in the analogous region as exon 33 were dependent on whether the channel had a short or long carboxy terminus (Murbartía et al. 2002). Alternative splicing might explain why two distinct sized isoforms of Ca\(_{\text{LVA}}\) channels were detected during Western blot studies of mouse brain (Yunker et al. 2003). Notably, these variants were found to vary in a tissue-specific and developmental pattern.

Chimeric studies between Ca\(_{\text{LVA}}\) and Ca\(_{\text{HVA}}\) identified a negatively charged region in the proximal carboxy terminus (23 amino acids after IVS6) as having an important role in channel inactivation (Staes et al. 2001). This region is conserved in all three Ca\(_{\text{LVA}}\) channels, suggesting that its role is conserved. Exon 33 splicing occurs 67 amino acid residues after IVS6, and the present results indicate this region has a greater role in Ca\(_{\text{LVA}}\) channel activation kinetics. Taken together, these results indicate that the carboxy termini of Ca\(_{\text{LVA}}\) channels modulate channel activity and may provide a site for binding of auxiliary subunits or posttranslational processing.

An unexpected finding of this study was that the effect of exon 9 splicing was highly dependent on the sequence at exon 33. Addition of exon 9 to channels containing the full exon 33 sequence had modest effects on channel gating, slowing recovery from inactivation and the voltage dependence of inactivation (comparison of \(-9 + 33\) vs. \(+9 + 33\)). These results indicate that exon 9 has little effect on the final transitions between open and inactivated states, acting instead on transitions between inactivated and closed states. In contrast, addition of exon 9 to channels containing the partial exon 33 sequence led to pronounced changes in channel activation and inactivation rates (comparison of \(-9Δ33\) vs. \(+9Δ33\)). Now exon 9 affected transitions between closed states and the open state and transitions from the open to inactivated state. Such interactions could result from two distinct mechanisms. One, gating transitions of domains I and IV may be transmitted allosterically, as they likely face each other in the three-dimensional structure. Or two, the intracellular loops interact directly, thereby modulating the effects the other has on gating.

We favor the second hypothesis based on the degree of structure-function conservation between LVA and HVA channels, and the physical interactions these loops have in Ca\(_{\text{LVA}}\) channels (Geib et al. 2002).
Recordings from neurons have revealed a large heterogeneity in the kinetics of T-type currents (Perez-Reyes 2003). Slow T-type currents have been described in neurons from thalamic reticular nucleus and lateral habenula, where they are thought to play a unique role in generating long bursts of firing (Huguenard et al. 1992, 1993). Because these regions also express abundant Ca$_{v}3.3$ mRNA and recombinant Ca$_{v}3.3$ channels display similar slow inactivation kinetics, it is likely...
that alternative splicing of CACNA1H plays an important role in these neurons (Talley et al. 1999). Subtype-specific antibodies might validate this hypothesis.

Modeling has provided a useful tool to infer the functional roles of ion channels in determining the firing patterns of neurons. Models of thalamic reticular and relay neurons have provided insight into the role of T-type Ca\(^{2+}\) channels in generating low-threshold spikes and how their kinetics, levels of expression, and distribution within the neuron can affect firing (Destexhe et al. 1996b; Huguenard and Prince 1994; McCormick and Huguenard 1992). Introduction of recombinant T-type channel parameters into these models confirms that fast inactivating channels such as Ca\(_{\text{v}3.1}\) and Ca\(_{\text{v}3.2}\) produce short spikes in response to depolarizing stimuli, whereas Ca\(_{\text{v}3.3}\) channels produce longer-lasting spikes that lead to sustained burst firing (Chemin et al. 2002). The present study shows that recombinant Ca\(_{\text{v}3.3}\) channel behavior can also be used to model rebound burst firing. Introduction of parameters for alternatively spliced variants indicated that these channels will produce distinct firing patterns. In general, the effect of alternative splicing is to decrease the ability of these channels to produce rebound spikes when compared with the predominant isoform (Δ9 + 33). One of the most critical determinants of rebound burst firing is the rate of activation. Channels that activate fast (e.g., Δ9 + 33) are capable of producing a LTS that triggers bursts that occur sooner and with more Na spikes in comparison to slowly activating channels (e.g., Δ9Δ33). Although a significant difference was noted in the rate of recovery from inactivation for the +9 + 33 channel, this difference did not appear to have much effect because varying the duration of the hyperpolarizing pulse did not alter the rank order of the channels to trigger firing. Channel availability is another important determinant of burst firing. A significant difference in the steady-state inactivation curve was noted for +9 + 33 channels, and this provides a likely explanation for why this variant triggered less firing after depolarizing pulses. Consistent with this hypothesis, all four variants behaved similarly to depolarizing pulses if the resting membrane potential was −85 mV. These results indicate alternative splicing modifies important channel transitions that determine neuronal excitability and that studies on T-type channel gating should focus more on the transitions that occur near the resting membrane potential of most neurons.

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GRANTS

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REFERENCES


Chemin J, Monteil A, Perez-Reyes E, Bourinet E, Nargeot J, and Lory P. Specific contribution of human T-type calcium channel isoforms (α_{1C}, α_{1H} and α_{1I}) to neuronal excitability. J Physiol 540: 3–14, 2002.


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