Alternative Splicing in the Voltage-Sensing Region of N-Type CaV2.2 Channels Modulates Channel Kinetics

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Lin, Yingxin, Stefan I. McDonough, and Diane Lipscombe. Alternative splicing in the voltage-sensing region of N-type CaV2.2 channels modulates channel kinetics. J Neurophysiol 92: 2820–2830, 2004. First published June 16, 2004; 10.1152/jn.00048.2004. The CaV2.2 gene encodes the functional core of the N-type calcium channel. This gene has the potential to generate thousands of CaV2.2 splice isoforms with different properties. However, the functional significance of most sites of alternative splicing is not established. The IVS3-IVS4 region contains an alternative splice site that is conserved evolutionarily among CaVα1 genes from Drosophila to human. In CaV2.2, inclusion of exon 31a in the IVS3-IVS4 region is restricted to the peripheral nervous system, and its inclusion slows the speed of channel activation. To investigate the effects of exon 31a in more detail, we generated four tsA201 cell lines stably expressing CaV2.2 splice isoforms. Coexpression of auxiliary CaVαβ and CaVαδ subunits was required to reconstitute currents with the kinetics of N-type channels from neurons. Channels including exon 31a activated and deactivated more slowly at all voltages. Current densities were high enough in the stable cell lines co-expressing CaV2.2 splice isoforms containing exon 31a correlated reasonably well with the properties of native N-type currents in sympathetic neurons. Our results suggest that alternative splicing in the S3–S4 linker influences the kinetics but not the voltage dependence of N-type channel gating.

IntroductioN

Calcium is a ubiquitous second messenger that regulates a wide range of cellular functions. In all excitable cells, voltage-gated calcium channels couple membrane depolarization to calcium entry. Functional and structural diversity among voltage-gated calcium channels is an important mechanism employed by cells to optimize calcium-dependent signaling. Ten genes encode the core CaVα1 subunits. Auxiliary CaVαβ and CaVαδ subunits and additional associated proteins modulate surface expression efficiency, subcellular targeting, biophysical properties, and pharmacology of the CaVα1 subunit (Birnbaumer et al. 1998; Herlon et al. 2002; Hering 2002; Walker and De Waard 1998).

Alternative splicing of CaVα1 pre-mRNAs is extensive, endowing individual CaVα1 genes with the capacity to generate multiple functionally specialized proteins (Lipscombe et al. 2002). The mammalian nervous system in particular uses alternative splicing to produce an array of functionally distinct and finely tuned proteins (Black 2000; Grabowski 1998). Sites of regulated alternative splicing occur in key functional domains of proteins (Garcia et al. 2004). The IVS3-IVS4 domain of CaVα1 is a conserved site of alternative splicing. It is found in multiple CaVα genes and different species from Drosophila to human (Barr et al. 1995; Bourinet et al. 1999; Hans et al. 1999; Ihara et al. 1995; Ligon et al. 1998; Lin et al. 1997, 1999; Lipscombe et al. 2002; Peixoto et al. 1997; Perez-Reyes et al. 1990; Smith et al. 1996, 1998; Snutch et al. 1991; Starr et al. 1991; Takimoto et al. 1997). Splice isoforms with different IVS3-IVS4 linkers can open at different rates and at different voltages and have different drug sensitivities (Bourinet et al. 1999; Hans et al. 1999; Krovetz et al. 2000; Lin et al. 1997, 1999; Lipscombe and Castiglioni 2004). The expression of exons in the IVS3-IVS4 region of at least two genes, CaV1.3 and CaV2.2, depends on tissue type (Lin et al. 1999; Takimoto et al. 1997). Evolutionary conservation, modification of channel function, and tissue-specific expression combine to suggest that alternative splicing in the IVS3-IVS4 region of CaVα is physiologically important.

In the CaV2.2 gene, exon 24a (e24a) encodes a tetrapeptide sequence, SFMG, in domain IIIIS3-IIIS4 and exon 31a (e31a), the dipeptide sequence, ET, in domain IVS3-IVS4 (Lipscombe et al. 2002). The expression pattern of these exons differs. In sympathetic neurons, all CaV2.2 mRNAs contain e31a, whereas this exon is absent in CaV2.2 mRNAs in brain and spinal cord. Exon e24a is present in an approximate reciprocal distribution. Most CaV2.2 mRNAs in brain and spinal cord contain e24a, whereas in sympathetic and dorsal ganglia a slight majority lack e24a (Lin et al. 1997). In previous studies, we characterized some aspects of alternative splicing in the IVS3–IVS4 linkers of the CaV2.2 channel in the Xenopus oocyte expression system. Our functional analyses in Xenopus oocytes showed that the peripheral-dominant CaV2.2[e24a, 31a] splice form activated more slowly and at voltages slightly more depolarized compared with the central-dominant form, CaV2.2[e24a, A31a] (Lin et al. 1997, 1999). Subsequently, we showed that the presence of e31a in domain IVS3-IVS4 accounts fully for the slower activation time course of CaV2.2[e24a, 31a] splice isoform, whereas both e24a and e31a contributed to the difference in activation thresholds (Lin et al. 1999).

In the present study, we generate mammalian cell lines (tsA201) stably expressing the N-type CaV2.2 splice forms...
with CaVβ3 alone or CaVβ3 and CaVαδ, the full complement of auxiliary subunits. We show that the presence of CaVαδ is necessary to reconstitute native-like ionic currents and confirm that CaV2.2 splice forms activate at different rates but find no difference in ionic activation thresholds as reported in previous studies using the oocyte expression system. Gating currents measured from heterologously expressed CaV2.2 splice forms decay with significantly different rates, but activation thresholds of gating currents are identical. Our analysis of ionic and gating currents in these stable cell lines correlates with native currents recorded from sympathetic and hippocampal pyramidal neurons. Our findings support the conclusion that alternative splicing in the S3-S4 linkers of CaV2.2 primarily serves to modify channel gating kinetics.

Note on nomenclature

In this paper, we use CaV2.2 for the alpha1 subunit of the N-type calcium channel (Ertel et al. 2000). There is no accepted nomenclature to identify splice isoforms. We know the structure of the human CaV2.2 gene (Lipscombe et al. 2002) and therefore use specific exon numbers to distinguish splice isoforms. The SFMG splice site in domain III3-III4 of CaV2.2 is encoded by exon 24a and the ET site in domain IVS3-IVS4 by exon 31a. The peripheral-dominant splice isoform studied here is CaV2.2 e[24a, 31a], formerly named mα1B-a, and the central-dominant splice isoform is CaV2.2 e[24a, 31a], formerly mα1B-c (Lin et al. 1997).

METHODS

Establishing stable lines

We generated four tsA201 cell lines that stably express the following calcium channel subunits. 1) CaV2.2 e[24a, 31a] and CaVβ3; 2) CaV2.2 e[24a, 31a] and CaVβ1; 3) CaV2.2 e[24a, 31a], CaVβ1, and CaVαδ; and 4) CaV2.2 e[24a, 31a], CaVβ3, and CaVαδ. All clones were generated in our laboratory. CaV2.2 subunits were subcloned into pcDNA6, blasticidin selection (Invitrogen). The two clones CaV2.2 e[24a, 31a] and CaV2.2 e[24a, 31a], originally called mα1B-a and mα1B-c (Lin et al. 1997) (GenBank No. AF055477), were linearized with SpeI. CaVβ3 was PCR-amplified from rat brain (Pan and Lipscombe 2000), subcloned into pcDNA3.1 (zeocin), and linearized by PvuII. CaVαδ was isolated from a rat superior cervical ganglia cDNA library (GenBank No. AF286488), subcloned into pcDNA3 (hygromycin), and linearized with SpeI (see http://neuroscience.brown.edu/LipscombeLab/HOMEPAGE/home2.htm for additional information on clones).

TABLE 1. Activation kinetics

<table>
<thead>
<tr>
<th>CaVαδ</th>
<th>Gating V 1/2</th>
<th>Ionic k1</th>
<th>Ionic k2</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.09 ± 0.02 (12)</td>
<td>0.26 ± 0.01 (6)</td>
<td>0.16 ± 0.02 (9)</td>
<td>0.01 ± 0.00 (13)</td>
</tr>
<tr>
<td>[24a, 31a]</td>
<td>0.11 ± 0.07 (16)</td>
<td>0.35 ± 0.03 (5)</td>
<td>0.28 ± 0.02 (10)</td>
</tr>
<tr>
<td>[32a, 31a]</td>
<td>0.12 ± 0.03 (6)</td>
<td>0.33 ± 0.00 (3)</td>
<td>0.26 ± 0.01 (8)</td>
</tr>
</tbody>
</table>

For clones, activation kinetics measured at 0 mV, deactivation kinetics at –60 mV with 2 Ba. Values are means ± SE. Numbers of neurons are in parentheses. For comparison, in Xenopus oocytes, activation kinetics for [24a, 31a] and [32a, 31a] were 2 ms and 4.5 ms at +10 mV with 5 Ba (Lin et al. 1997). *For native N-type, activation kinetics measured at +10 mV and deactivation kinetics measured at –50 mV with 5 Ba.
Two cell lines were initially generated that stably expressed one of the two CaV2.2 splice forms together with CaVβ3, tsA201 cells were transfected by electroporation with a 10 μg mix of linearized CaV2.2 and CaVβ3 cDNAs. Cells were diluted and grown without antibiotics for 2 days; 7.5 μg/ml blasticidin and 250 μg/ml zeocin were then added and single colonies isolated 10 days later. Selection with blasticidin and zeocin was carried out, and 20 new colonies were chosen randomly for functional screening by whole cell recording. A second round of dilution cloning, PCR screening, and two cell lines that contained high current density were chosen for functional assessment. For example, 20 of 20 colonies contained CaV2.2 e[Δ24a, 31a] and 18 of 20 contained CaVβ3 based on PCR screening. For each condition, six cell lines were chosen randomly for functional screening by whole cell recording, and two cell lines that contained high current density were selected. There was significant variability in current density among cells in each line. A second round of dilution cloning, PCR screening, and functional assessment was carried out, and 20 new colonies were isolated for each subunit combination. Cell lines 2017 (CaV2.2 e[Δ24a, 31a], CaVβ3) and 191105 (CaV2.2 e[24a, Δ31a], CaVβ3, CaVα,δ1) were used for further study. We fully sequenced all CaV2.2 cDNAs from these cell lines to ensure that no random mutations were introduced.

Recording methods

All recordings were carried out at room temperature (22–23.5°C). Currents were recorded using the whole cell recording method (Axopatch 200A), digitized at 10 kHz, and low-pass filtered at frequencies between 2 and 5 kHz. Analysis was performed with pCLAMP7 (Axon Instruments), software was custom-written in Quick Basic or MatLab, and Origin (Microcal). The bath solution contained (in mM) 160 TEA-Cl, 10 HEPES, and 2 BaCl2, pH 7.4 with TEA-OH. The pipette solution contained (in mM) 56 CsCl, 68 CsF, 2.2 MgCl2, 4.5 EGTA, 9 HEPES, 4 MgATP, 14 creatine phosphate (Tris salt), and 0.3 GTP (Tris salt), pH 7.4 with CsOH. Electrode resistances were 1–2 MΩ when filled with pipette solution. The series resistance was always <10 MΩ and compensated >90%. Currents evoked by voltage steps were subtracted for leak and capacitance using appropriately scaled currents evoked by a hyperpolarization from the holding potential. α-conotoxin GVIA (Peptides International or Bachem) was used to isolate N-type currents from whole cell calcium currents in sympathetic and hippocampal neurons.
Gating currents

Gating currents were resolved in cells expressing Ca_{\alpha 2}\delta together with Ca_{\alpha} and Ca_{\alpha2}\beta. Currents were sampled at 10 kHz and filtered at 5 kHz; twice the digitization frequency to avoid aliasing. At 5 kHz, the rise time of events is 66.42 µs. For event amplitudes to be measured reliably, durations should be at least two times t_i or 132 µs (Colquhoun 1994). It is possible that our fastest signals (160 µs for gating of central dominant isoform) were slightly undersampled and amplitudes underestimated. However, these were clearly distinguishable from the slower gating currents of the peripheral dominant isoform (τ = 280 µs) that were well resolved. Nonlinear charge movements were recorded in barium with steps directly to the ionic reversal potential. The reversal potential was determined for each cell and was between +55 and +65 mV. Currents were induced by step depolarizations from a holding potential of between +100 and –120 mV. Leak subtraction was carried out off-line with appropriately scaled currents evoked by a 10 or 20 mV hyperpolarization from the holding potential. Recordings were only made from cells with small leak currents, in the range of 10–50 pA at the holding potential (–100 or –120 mV). Small transient outward currents remained after leak subtraction of size in relation to ionic currents appropriate for gating currents (Jones et al. 1997–1999; Noceti et al. 1996). No nonlinear charge movements were recorded from cells expressing no or very few functional channels. Similar nonlinear charge movements were recorded at the onset and offset of test depolarizations when ionic currents were blocked by replacing 2 mM Ba^{2+} with 2 mM Co^{2+} and 0.2 mM Cd^{2+}.

RESULTS

Ca_{\alpha 2}\delta subunit modifies channel gating kinetics

N-type Ca channel currents were recorded from mammalian tsA201 cells stably expressing Ca_{\alpha} [24a, 31a] together with Ca_{\alpha} and Ca_{\alpha2}\beta (Fig. 1). For both splice forms, gating kinetics of the channels in tsA201 cells were significantly faster than those of the same clones expressed in Xenopus oocytes with Ca_{\alpha} (Lin et al. 1997) and resembled N-type currents of neurons (see following text; Table 1). At least part of the explanation for faster and more native-like gating kinetics of Ca_{\alpha 2} clones in tsA201 cells is the presence of exogenous Ca_{\alpha2}\delta_1 auxiliary subunit was...
Xenopus oocytes (Lin et al. 1997), compared with 1.6 and 0.7 ms in tsA201 cells in the absence and presence of CaVαδ, respectively (Table 1). Activation curves generated from tail current analysis in cells expressing exogenous CaVαδ were also steeper at voltages between −30 and +30 mV compared with those generated from cells lacking CaVαδ and generally contained two Boltzmann components rather than one (Figs. 1B, 2F, and 5A). We conclude that the presence of CaVαδ is an important determinant of channel kinetics, accounting for the faster, more native-like kinetics of currents in tsA201 cells that express exogenous CaVαδ.

Gating current measurements

Current densities and therefore channel expression levels were sufficiently high in these stable cell lines to resolve gating charge movement: about nine elementary charges per channel (Noceti et al. 1996). We recorded gating currents using identical ionic conditions as those used to record ionic currents (Figs. 2, 4, and 5). We applied test pulse depolarizations to precisely the ionic reversal potential where net current is zero. After leak subtraction only small, transient currents remained that decayed with time constants in the range of 0.2–0.3 ms, consistent with the time course of ON gating currents (Fig. 2, A, B, and D) (Jones et al. 1997, 1999; Noceti et al. 1996). Figure 2 shows that the kinetics of these gating currents were the same when measured in barium at the ionic reversal potential (+60 mV) and when measured in the same cell with ionic currents blocked by a combination of cobalt (2 mM) and cadmium (0.2 mM) (compare Fig. 2, A and B, and see D). The time course of the gating current during the test pulse, representing the speed of ON movement of the charge sensor, was fit well by a single exponential function ($\tau = 0.36$ ms for this cell expressing CaV2.2α[Δ24α, 31α]; Fig. 2D). As a control, we also show that the total charge during ON gating currents equals that of OFF gating currents, over a range of potentials (Fig. 2, B and E). The size of ON gating currents at the reversal potential diminished as the magnitude of the prepulse depolarization immediately preceding the step to +60 mV was increased (Fig. 2, A and C). This is expected for current originating from the movement of putative voltage sensors. As progressively more channels activate and as more gating charge moves during the prepulse, a proportionally smaller number of channels is available for activation and less gating charge moves during the subsequent test pulse. The total charge left to move at +60 mV as a function of prepulse voltage is shown in Fig. 2C. The inverse of this plot is the ON gating current activation curve (Figs. 2F and 5B). As expected, gating currents activate at voltages more hyperpolarized than those that elicit ionic currents (~40 mV; Figs. 2F, and 5, C and D, also see Table 1). However, we found that gating currents in the presence of cobalt and cadmium required much stronger depolarizations to activate (~25 mV more depolarized) compared with those recorded in barium (Fig. 2F). Differences in surface charge screening between barium and cobalt may account for the differential shifts in the voltage dependence of channel gating (Hille et al. 1975). To avoid the need to correct for surface charge screening and to facilitate direct comparisons between gating and ionic currents, we measured gating currents at the ionic reversal potential in the presence of 2 mM barium.
Alternative splicing in S3-S4 modifies ionic current kinetics

We compared N-type currents recorded from tsa201 cells stably expressing each Ca\textsubscript{v}2.2 splice isoform. Macroscopic Ca\textsubscript{v}2.2ε[Δ24a, 31a] currents activated and deactivation with time constants that were significantly longer compared with Ca\textsubscript{v}2.2ε[24a, Δ31a] (Fig. 3, C and D; P < 0.05; Table 1). That is, the predicted peripheral-dominant clone activated and deactivated more slowly than the predicted central-dominant clone. The difference in activation speed was ~1.5-fold over a range of voltages. This difference in macroscopic activation kinetics was also independent of the presence of Ca\textsubscript{v}2.2εδ3 (Table 1) and matches almost exactly our previous studies of the same splice isoforms in Xenopus oocytes (Lin et al. 1997, 1999).

Gating currents of S3-S4 splice forms decay at different rates

We compared gating currents from cells expressing Ca\textsubscript{v}2.2 splice isoforms as described earlier. Gating currents measured in cells expressing Ca\textsubscript{v}2.2 e[Δ24a, 31a] decayed with time courses that were significantly slower (1.7-fold) compared with Ca\textsubscript{v}2.2 e[24a, Δ31a] (P < 0.05; Fig. 4; Table 1). The 1.7-fold difference parallels the different gating kinetics of ionic currents between splice forms (Figs. 3C; Table 1). Although gating currents were measured at +60 mV, ionic currents were measured at voltages between −10 and +30 mV. Our results suggest that alternative splicing in IVS3-IVS4 linker in Ca\textsubscript{v}2.2 modifies the rate of movement of the voltage sensor that precedes channel opening.

Steady-state voltage dependence of activation does not differ between isoforms

Next we analyzed the steady-state voltage dependence of activation of ionic and gating currents. In our previous studies, we reported a small (~7 mV) but significant difference in the voltage dependence of channel activation between splice isoforms expressed in oocytes. This difference was not recapitulated in mammalian cells (Table 1; Fig. 5A). Most ionic current activation curves (8 of 10 recordings) were fit by two Boltzmann functions with activation mid-points close to 0 and +45 mV, respectively (Figs. 2F and 5A; Table 1). There was no consistent difference in tail current activation curves between splice isoforms. In contrast to ionic currents, activation curves of gating
Many factors that affect the behavior of N-type channels including Ca$_{v}$$beta$ subunits and G protein modulation are likely to differ in different types of neurons (Ikeda and Dunlap 1999; Walker and De Waard 1998). However, our analyses of cloned splice isoforms, together with earlier studies reporting tissue-specific expression of alternatively spliced exons in Ca$_{v}$$alpha_2$, make predictions about how native N-type currents in different regions of the nervous system might differ (Lipscombe et al. 2002). The vast majority of N-type currents of sympathetic neurons contain the e31a-containing Ca$_{v}$$alpha_2$ isoform. These N-type currents should activate and deactivate with slower kinetics compared with central neurons that only express Ca$_{v}$$alpha_2$ isoforms lacking e31a. In addition, our analyses in tsA201 cells suggest no significant difference in the voltage-dependence of N-type channel activation between sympathetic (peripheral) and hippocampal (central) neurons.

To compare N-type currents in sympathetic and pyramidal hippocampal neurons, we isolated currents using $omega$-conotoxin GVIA-subtraction because $omega$-conotoxin GVIA inhibits both Ca$_{v}$$alpha_2$ splice isoforms equally well. The N-type current represents $sim$80% of the whole cell calcium channel current in sympathetic neurons and $sim$20% in hippocampal neurons (Fig. 7). When we compared the voltage dependencies of activation, we found no significant difference between native N-type currents in sympathetic and hippocampal neurons, consistent with our studies in tsA201 cells (Fig. 8, A and B; Table 1). There was no obvious difference in the time course of inactivation of N-type currents between these cell types, at least during the 20-ms test depolarizations used here (Lin et al. 1999). However, N-type currents isolated from recordings of sympathetic neurons both activated and deactivated with kinetics that were consistently slower than N-type currents recorded from hippocampal neurons (Fig. 8, C and D; Table 1; $P < 0.05$). In fact, deactivation kinetics of native N-type currents in sympathetic and hippocampal neurons were indistinguishable from Ca$_{v}$$alpha_2$ e[A24a, 31a] and Ca$_{v}$$alpha_2$_e[A24a, $delta_3$] splice isoforms expressed in tsA201 cells, respectively (Fig. 7D; Table 1). We found that activation kinetics of native N-type currents were somewhat slower than those of Ca$_{v}$$alpha_2$ e[A24a, 31a] expressed in tsA201 cells (Table 1). Although not a perfect match, overall, the time course and voltage depen-
dence of channel opening of native N-type channel currents are most similar to the properties of CaV2.2 splice isoforms expressed in tsA201 cells that also express CaVα2δ1 (Figs. 3–5; Table 1). Our results from recordings of cloned and native N-type currents suggest that the major consequence of alternative splicing in the S3-S4 linkers of CaV2.2 is modulation of channel gating kinetics and not steady-state voltage dependence.

**Discussion**

We have analyzed the biophysical properties conferred on the N-type channel by two short exons in CaV2.2 that are differentially expressed in central and peripheral regions of the nervous system (Lin et al. 1997). This form of alternative splicing is characterized by inverse insertions of short cassette exons in domains III-S3-IIS4 and IVS3-IVS4. Results show that the CaV2.2e[P24a, Δ31a] splice isoform gates more rapidly compared with CaV2.2e[Δ24a, 31a] over a range of voltages, independent of expression system and of the presence of the CaVα2δ auxiliary subunit. Steady-state voltage dependencies of channel opening and of charge movement were not different between splice forms when expressed in mammalian cells. Data from gating currents provide direct evidence that the putative voltage sensor of the CaV2.2e[P24a, Δ31a] splice isoform moves faster compared with CaV2.2e[Δ24a, 31a].

Biophysical differences between these splice forms are likely to be physiologically significant because expression of the two exons is under tight cellular control. More generally, alternative splicing, particularly in the IVS3-IVS4 linker, is conserved among CaVα2δ genes (Lipscombe et al. 2002). In support of this view we show that native N-type currents recorded in hippocampal neurons reflect the faster kinetics of the CaV2.2e[P24a, Δ31a] splice isoform, whereas N-type currents in sympathetic neurons consistently gate more slowly similar to CaV2.2e[Δ24a, 31a]. Clearly, other factors will contribute to setting the time course of calcium channel activation and deactivation, including G protein activation and association with different CaVαβ subunits (Ikeda and Dunlap 1999; Walker and De Waard 1998), and these may differ between sympathetic and hippocampal neurons. CaV2.2 has been shown to associate with different CaVαβ subunits in vivo (Scott et al. 1996). However, we only observed significant differences in activating and deactivating kinetics of native N-type currents in sympathetic and hippocampal neurons consistent with the expression pattern of CaV2.2 splice isoforms. Exon e31a of CaV2.2 is particularly notable because it is expressed in peripheral neurons but suppressed throughout the CNS, including the hippocampus. Further, in a previous study we showed that exon 31a can fully confer the slower activation of the CaV2.2e[Δ24a, 31a] splice isoform (Lin et al. 1999). The different kinetics of ionic and gating currents of CaV2.2 splice isoforms studied here most likely originate from the splice site in the S3-S4 linker of the fourth domain.

**Effects on gating kinetics**

The slower gating kinetics of CaV2.2e[Δ24a, 31a] reported here and in earlier studies (Lin et al. 1997, 1999) are consistent with studies of alternative splicing in the equivalent IVS3-IVS4 linker of the closely related CaV2.1 P/Q-type channel (Hans et al. 1999; Krovetz et al. 2000). The different phenotypes of S3-S4 splice isoforms of CaVα2δ are interesting in light of the proposed central role this region plays in forming the putative voltage-sensing paddles recently predicted from crystallographic studies of the voltage-gated potassium channel (Jiang et al. 2003; see also Bezanilla 2000; Nakai et al. 1994; Stotz and Zamponi 2001). Apparent rates of activation of macroscopic currents, deactivation rates of macroscopic tail currents, and the speed of on gating currents all differ between splice forms, with the CNS-dominant CaV2.2e[+24a, Δ31a] channels faster by ~1.5-fold in all cases (Figs. 3 and 4; Table 1). We note, however, that gating currents were monitored at or close to +60 mV, whereas ionic currents were recorded at voltages between −100 and +30 mV where signals are measurable (Fig. 3). Several schemes modeling the kinetics of
activation of voltage-gated channels, including calcium channels, invoke a final voltage-independent conformational closed-open step that is rate limiting at sufficiently strong depolarizations (Chen and Hess 1990; Frazier et al. 2001; Zagotta et al. 1988). We observed kinetic differences between S3 and S4 splice isoforms over a range of voltages while steady-state activation curves for both gating and ionic currents were not distinguishable between isoforms. These observations are consistent with the involvement of a gating transition closely linked to the open state perhaps in the final voltage-independent closed-open transition.

Summary

The mammalian CaV2.2 gene is encoded by ≥50 exons, ≥10 of which can be alternatively spliced. In addition to exons 24a and 31a, alternative splicing involves exon 10 in the I–II intracellular loop, exons 18a, 19, 20, and 21 in the II–III intracellular loop, and exons 37a/37b, and 46 in the C terminus (Lipscombe and Castiglioni 2004). It is therefore important to note that there is no one peripheral or central form of the CaV2.2 gene. Indeed, our recent single-cell RT-PCR analysis shows that even individual neurons of the dorsal root ganglia express multiple CaV2.2 splice isoforms (Bell et al. 2004). Significantly, however, of the 10 sites of alternative splicing that have been identified so far in mammalian CaV2.2 genes (Lipscombe and Castiglioni 2004), only exon 31a is restricted to peripheral neurons; based on our recent single-cell RT-PCR analysis in dorsal root ganglia, exon 31a is present in every CaV2.2 mRNA (Bell et al. 2004). Collectively, our studies are therefore consistent with the conclusion that tissue-specific expression of exon 31a of CaV2.2 underlies the comparatively slow kinetics of N-type calcium channels in peripheral neurons. Pharmacological tools to discriminate between these splice isoforms would help establish their relative contribution to synaptic events. The Conus peptides tested here did not differentiate between splice isoforms, although others have reported differential effects of Conus catus, peptides under...
certain conditions (Lewis et al. 2000). The voltage dependence of the two splice forms in mammalian cells are equal, therefore pharmacological strategies to distinguish between them would probably need to exploit the different binding epitopes in the S3-S4 linkers directly. The cell lines developed here provide useful tools for studying splice isoforms of the N-type Ca channel that exhibit properties similar to those of native currents (Barrett et al. 2001) and with current densities sufficiently high to monitor gating currents.

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