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L-Type Calcium Channels: The Low Down

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Lipscombe, Diane, Thomas D. Helton, and Weifeng Xu. L-type calcium channels: The low down. *J Neurophysiol* 92: 2633–2641, 2004; 10.1152/jn.00486.2004. L-type calcium channels couple membrane depolarization in neurons to numerous processes including gene expression, synaptic efficacy, and cell survival. To establish the contribution of L-type calcium channels to various signaling cascades, investigators have relied on their unique pharmacological sensitivity to dihydropyridines. The traditional view of dihydropyridine-sensitive L-type calcium channels is that they are high-voltage–activating and have slow activation kinetics. These properties limit the involvement of L-type calcium channels to neuronal functions triggered by strong and sustained depolarizations. This review highlights literature, both long-standing and recent, that points to significant functional diversity among L-type calcium channels expressed in neurons and other excitable cells. Past literature contains several reports of low-voltage–activated neuronal L-type calcium channels that parallel the unique properties of recently cloned $Ca_V1.3$ L-type channels. The fast kinetics and low activation thresholds of $Ca_V1.3$ channels stand in stark contrast to criteria currently used to describe L-type calcium channels. A more accurate view of neuronal L-type calcium channels encompasses a broad range of activation thresholds and recognizes their potential contribution to signaling cascades triggered by subthreshold depolarizations.

L-type calcium channels regulate numerous neuronal functions

L-type calcium channels are perhaps the best characterized of the voltage-gated calcium channels. They were first recognized as essential for coupling excitation to contraction in skeletal, cardiac, and smooth muscle cells (Beam et al. 1989; Franzini-Armstrong and Protasi 1997; Reuter 1985; Schneider and Chandler 1973; Tanabe et al. 1990). L-type calcium channels are also expressed in neurons and endocrine cells where they regulate a multitude of processes including secretion of neurohormones and transmitters, gene expression, mRNA stability, neuronal survival, ischemic-induced axonal injury, synaptic efficacy, and the activity of other ion channels (Ashcroft et al. 1994; Bading et al. 1993; Bean 1989; Charles et al. 1999; Christie et al. 1997; De Koninck and Cooper 1995; Deisseroth et al. 1998; Dunlap et al. 1995; Finkbeiner and Greenberg 1998; Fuchs 1996; Galli et al. 1995; Heidelberger and Matthews 1992; Kamsler and Segal 2003; Lei et al. 2003; Marrion and Tavalin 1998; Marshall et al. 2003; Murphy et al. 1991; Norris et al. 1998; Ouardouz et al. 2003; Sand et al. 2001; Schorge et al. 1999; Shinnick-Gallagher et al. 2003; Smith et al. 1993; Tachibana et al. 1993; Thaler et al. 2001; Thibault et al. 2001; Weisskopf et al. 1999; Wiser et al. 1999; Zhang and Townes-Anderson 2002). The unique pharmacological sensitivity of L-type calcium channels to dihydropyridine agonists and antagonists has proved critical for their identification in physiological assays and also for their biochemical isolation (Kanngiesser et al. 1988). Biochemical purification of the dihydropyridine receptor from skeletal muscle was the essential step in cloning the first voltage-gated calcium channel (Tanabe et al. 1987). Sequence information from this landmark study was then used to screen for and clone $Ca_V1.2$ and $Ca_V1.3$ cDNAs (Biel et al. 1990; Hui et al. 1991; Koch et al. 1990; Mikami et al. 1989; Perez-Reyes et al. 1990; Williams et al. 1992). Functional analyses of cloned channels, primarily of $Ca_V1.2$, were generally consistent with native cardiac L-type channels and the following criteria evolved for their identification.

1) Activation by strong depolarizations (high-voltage–activated [HVA]).

2) High sensitivity to dihydropyridine agonists and antagonists.

3) Relatively slow activation kinetics.

4) Calcium-dependent inactivation with little voltage-dependent inactivation (*long-lasting*).

5) Large single-channel conductance.

However, a substantial body of evidence points to heterogeneity among neuronal L-type calcium channels that has until recently received little attention. This review highlights recent studies of cloned channels, as well as long-standing studies of native L-type channels, that point to significant deviations from criteria listed above in the properties of L-type channels.

Ca_V1 genes encode L-type calcium channels

Identifying the genes that encode core $Ca_V\alpha_1$ subunits of voltage-gated calcium complexes has led to a comprehensive sequence-based classification scheme (Fig. 1). When sequences are compared, voltage-gated calcium channels fall into three main groups: Ca_V1 (L-type), Ca_V2 (P-type, N-type, and R-type), and Ca_V3 (T-type) (Ertel et al. 2000; Lipscombe 2002b). In general, these gene families correspond to the subtypes of calcium channels defined by functional and pharmacological criteria. Ca_V1 and Ca_V2 genes are more closely related to each other when compared with Ca_V3 genes. Ca_V3 T-type channels possess certain functional properties that set them apart from other voltage-gated calcium channels (Perez-Reyes et al. 1998). Low-voltage–activating calcium current typically marks the presence of Ca_V3 T-type channels. However, as we will discuss, this property is shared by $Ca_V1.3$, a member of the Ca_V1 gene family, and thus should not be considered unique to Ca_V3 (Avery and Johnston 1996; Koschak et al. 2001; Lipscombe 2002a; Platzer et al. 2000; Scholze

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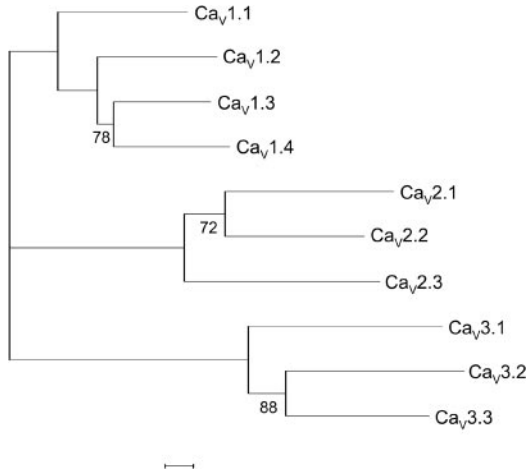

FIG. 1. $Ca_V\alpha_1$ subunit gene tree. Full-length amino acid sequences for all 10 human $Ca_V\alpha_1$ genes were aligned using a branch and bound tree search with maximum parsimony (Genetic Computer Group, paupsearch and paupdisplay programs). Accession numbers for sequences used are: $Ca_V1.1$, L33795; $Ca_V1.2$, AJ224873; $Ca_V1.3$, M76558; $Ca_V1.4$, AJ224874; $Ca_V2.1$, AB035727; $Ca_V2.2$, M94173; $Ca_V2.3$, L27745; $Ca_V3.1$, AF190860; $Ca_V3.2$, AF05196; $Ca_V3.3$, AF211189. Confidence values for each node were determined by bootstrap analysis. All unlabeled nodes represent 100% confidence. Representation was rooted using the midpoint method. Scale bar represents 1 substitution per 100 amino acids. This tree is essentially the same when we exclude the variable intracellular loops (I–II, II–III) and N- and C-termini (Lipscombe 2002b). Our analysis indicates a stronger similarity between $Ca_V1.3$ and $Ca_V1.4$ genes compared with that of other published Ca_V gene trees (Ertel et al. 2000). If we just align sequences of the 4 Ca_V1 genes the confidence value at the $Ca_V1.3$–$Ca_V1.4$ node increases to 100%.

et al. 2001; Xu and Lipscombe 2001). Until recently, all members of Ca_V1 and Ca_V2 gene families were considered high-voltage–activated. Ca_V1 channels are distinguishable from Ca_V2 channels primarily by their unique pharmacology. Ca_V1 channels are sensitive to dihydropyridine agonists and antagonists, but are not blocked by ω-aga IVA or ω-conotoxin GVIA, which inhibit $Ca_V2.1$ and $Ca_V2.2$ channels, respectively (Bean 1991; Cruz et al. 1987; McIntosh et al. 1999; Mintz et al. 1992). However, as we will discuss, dihydropyridine antagonists do not completely inhibit all L-type channels.

Four mammalian Ca_V1 genes encode L-type calcium channels

Four Ca_V1 genes are present in the human genome, referred to as $Ca_V1.1$–1.4 (Fig. 1). The $Ca_V1.1$ gene, formerly α_{1S}, is expressed in skeletal muscle. $Ca_V1.1$ directly links to ryanodine receptors in the sarcoplasmic reticulum (Flucher and Franzini-Armstrong 1996). $Ca_V1.1$ primarily acts as a voltage sensor, coupling depolarization to release of intracellular calcium by activating the ryanodine receptor. The influx of calcium through the ion pore of $Ca_V1.1$ during gating is secondary to its primary role as a voltage sensor (Schwartz et al. 1985). The coupling between depolarization and channel opening is inefficient; $Ca_V1.1$ channels open with slow kinetics (Almers and Palade 1981; Rios and Brum 1987; Tanabe et al. 1988).

The $Ca_V1.2$ gene, formerly α_{1C}, is expressed in a variety of cells including ventricular cardiac muscle, smooth muscle, pancreatic cells, fibroblasts, and neurons (Diebold et al. 1992; Koch et al. 1990; Mori et al. 1993; Perez-Reyes et al. 1990; Schultz et al. 1993; Soldatov 1992; Takimoto et al. 1997; Welling et al. 1997). This channel opens as the membrane potential depolarizes beyond about −30 mV. $Ca_V1.2$ channels help define the shape of the action potential in cardiac and smooth muscle. These channels function primarily as calcium ion channels and, unlike $Ca_V1.1$ of skeletal muscle, calcium flow through $Ca_V1.2$ is an essential step in initiating the signaling cascade that leads to cardiac and smooth muscle contraction (Reuter et al. 1988; Tanabe et al. 1990). In neurons $Ca_V1.2$ channels are thought to couple membrane depolarization to regulation of gene expression (Dolmetsch et al. 2001; Weick et al. 2003).

The $Ca_V1.3$ gene, formerly α_{1D}, is expressed in many of the same cells that express $Ca_V1.2$. In neurons, $Ca_V1.2$ and $Ca_V1.3$ are often found in the same general neuronal compartments, particularly dendrites, but their subcellular distributions appear distinct (Hell et al. 1993; Westenbroek et al. 1998). $Ca_V1.3$ has been found to co-localize with the small conductance calcium-activated potassium channel (Bowden et al. 2001). $Ca_V1.3$ is also expressed in pancreatic beta cells, neuroendocrine cells, photoreceptors, amacrine cells, and hair cells of the inner ear where it mediates synaptic transmission (Habermann et al. 2003; Ihara et al. 1995; Kollmar et al. 1997a,b; Liu et al. 2004; Morgans 1999; Morgans et al. 1998; Platzer et al. 2000; Russo et al. 2003; Safa et al. 2001; Scholze et al. 2001; Seino et al. 1992; Taylor and Morgans 1998). In the heart, $Ca_V1.3$ is present in atrial tissue where it contributes to pacemaking (Mangoni et al. 2003; Platzer et al. 2000; Takimoto et al. 1997; Zhang et al. 2002), but not in ventricular muscle that expresses $Ca_V1.2$.

The $Ca_V1.4$ gene, formerly α_{1F}, is expressed primarily in retina and is linked to a rare human disorder, stationary night blindness (Bech-Hansen et al. 1998; Strom et al. 1998). $Ca_V1.4$ is found at synaptic terminals of retinal bipolar cells, and RNA encoding $Ca_V1.4$ has also been PCR amplified from dorsal root ganglia (Berntson et al. 2003; Murakami et al. 2001). Interestingly, the $Ca_V1.4$ gene sequence is more homologous to $Ca_V1.3$, based on comparisons among available Ca_V clones (Fig. 1).

L-type calcium channels are functionally diverse

Analyses of Ca_V1 clones in various heterologous expression systems have provided compelling data that L-type calcium channels are a functionally heterogeneous family.

1) Not all L-type calcium channels require strong depolarizations for activation; $Ca_V1.3$ and $Ca_V1.4$ channels have low activation thresholds.

2) L-type calcium channels are not all inhibited equally well by dihydropyridine antagonists; $Ca_V1.3$ and $Ca_V1.4$ L-type channels are significantly less sensitive compared with $Ca_V1.2$.

3) Activation kinetics of L-type calcium channels vary. $Ca_V1.3$ channels activate with fast kinetics, whereas $Ca_V1.1$ channels open slowly.

4) Certain $Ca_V1.4$ L-type channels do not exhibit calcium-dependent inactivation. Furthermore, in physiological solutions that contain calcium, L-type calcium channels that do undergo calcium-dependent inactivation are not *long lasting*.

5) Most L-type calcium channels have relatively large single-channel conductances when isotonic barium is the charge

carrier. However, analyses of single $Ca_V1.3$ and $Ca_V1.4$ channels are lacking.

Skeletal muscle $Ca_V1.1$ and cardiac $Ca_V1.2$ L-type channels are functionally distinct

Although the first $Ca_V\alpha_1$ subunit to be cloned, $Ca_V1.1$ resisted functional reconstitution in nonmuscle, heterologous expression systems (Tanabe et al. 1987). Successful expression of $Ca_V1.1$ was eventually achieved using embryonic muscle from dysgenic mice. In a series of classic experiments, Numa, Beam, and colleagues, rescued excitation–contraction coupling as well as L-type calcium channel currents in dysgenic muscle by expressing $Ca_V1.1$ cDNA in these cells (Adams et al. 1990; Tanabe et al. 1988). $Ca_V1.1$ currents were small and activated with slow kinetics, properties consistent with native L-type currents in skeletal muscle. As in skeletal muscle, contraction depended on the mobilization of intracellular calcium stores. $Ca_V1.2$ could also reconstitute excitation–contraction coupling in dysgenic muscle, but the features were distinctly cardiac-like. In this case, calcium flux across the membrane through $Ca_V1.2$ channels was essential to trigger muscle contraction (Tanabe et al. 1990). $Ca_V1.2$ channels opened with rates that were significantly faster compared with $Ca_V1.1$. Chimeric analyses demonstrated that sequence differences in the II–III intracellular linker region of $Ca_V1.1$ and $Ca_V1.2$ genes imparted the skeletal or cardiac muscle form of excitation–contraction coupling. In subsequent experiments Beam and colleagues demonstrated that sequence differences in the domain IS3–IS4 linkers of $Ca_V1.1$ and $Ca_V1.2$ genes determined the gating phenotypes of these two L-type calcium channels (Nakai et al. 1994). The slow gating phenotype of $Ca_V1.1$ channels was transferred to $Ca_V1.2$ by swapping in the IS3–IS4 linker of $Ca_V1.1$. Variations in S3–S4 linker sequences among other voltage-gated ion channel gene families and their splice isoforms are similarly important in modulating channel gating kinetics (Lipscombe 2002b; Mathur et al. 1997; Tang and Papazian 1997).

$Ca_V1.2$ and $Ca_V1.3$ L-type channels are functionally distinct

With the exception of skeletal muscle and perhaps retina, all excitable cells express one or both $Ca_V1.2$ and $Ca_V1.3$ genes. The products of these genes constitute the major fraction of L-type calcium channels in mammals (Hell et al. 1993; Ludwig et al. 1997; Takimoto et al. 1997; Williams et al. 1992). Until recently, the prevailing image of the neuronal L-type calcium channel was of a high-voltage-activated, slowly activating channel with high sensitivity to dihydropyridines (Ertel et al. 2000; Hille 2001). These features have developed primarily from biophysical analyses of heterologously expressed $Ca_V1.2$ L-type calcium channels (Altier et al. 2001; Bourinet et al. 1994; Charnet et al. 1994; de Leon et al. 1995; Ivanina et al. 2000). Although $Ca_V1.3$ was first cloned in the early 1990s, low expression levels in heterologous systems limited electrophysiological studies of this L-type calcium channel (Hui et al. 1991; Williams et al. 1992). Aside from a report that $Ca_V1.3$ L-type channels could be reversibly inhibited by the N-type calcium channel blocker ω-conotoxin GVIA (Williams et al. 1992), a result that has not been confirmed (Xu and Lipscombe 2001), $Ca_V1.3$ channels were not considered unique.

The $Ca_V1.3$ knockout mice, however, renewed interest and provided compelling evidence that the $Ca_V1.3$ gene encodes L-type calcium channels with unusual properties (Mangoni et al. 2003; Platzer et al. 2000; Zhang 2002). At the behavioral level, mice lacking the L-type $Ca_V1.3$ gene experience significant sinoatrial node dysfunction characterized by sinus bradycardia. This unanticipated role for $Ca_V1.3$ in pacemaking implies that these L-type calcium channels are important in mediating subthreshold depolarizations in the sinoatrial node. The absence of a low-threshold activating calcium current in sinoatrial node cells of $Ca_V1.3$ $-/-$ mice confirmed this hypothesis (Zhang 2002). Hearing loss and the absence of a low-threshold activating calcium current in hair cells from these mice are consistent with prominent expression of $Ca_V1.3$ in inner hair cells of the cochlea (Kollmar et al. 1997b; Platzer et al. 2000). The functional properties of $Ca_V1.3$ clones isolated from neurons and endocrine cells, more recently, confirm that $Ca_V1.3$ L-type channels activate at subthreshold voltages (Koschak et al. 2001; Platzer et al. 2000; Safa et al. 2001; Xu and Lipscombe 2001).

$Ca_V1.3$ L-type channels activate at relatively hyperpolarized membrane potentials

Figure 2 illustrates that $Ca_V1.2$ and $Ca_V1.3$ channels have very different activation thresholds. The $Ca_V1.2$ and $Ca_V1.3$ clones used were isolated from neuronal tissue, expressed in tsA201 cells, and recorded under identical conditions. With physiological concentrations of extracellular calcium, $Ca_V1.3$ channels start to activate at about -55 mV, a voltage that is approximately 20–25 mV more hyperpolarized as compared with $Ca_V1.2$. Low-threshold activation is a prominent feature of all $Ca_V1.3$ clones isolated recently, independent of tissue of origin and of auxiliary subunits (Koschak et al. 2001; Safa et al. 2001; Scholze et al. 2001; Xu and Lipscombe 2001). Why was this unique and salient feature of $Ca_V1.3$ not highlighted in earlier studies (Bell et al. 2001; Ihara et al. 1995; Williams et al. 1992)? The most likely explanation relates to the use of high concentrations of extracellular barium and calcium in these studies to compensate for low expression levels. Under these conditions, $Ca_V1.3$ channels would have activated at significantly more depolarized voltages as a result of surface charge screening (Frankenhaeuser and Hodgkin 1957; Hille 2001). Indeed, when we studied $Ca_V1.3$ L-type currents under similar conditions, 40 mM extracellular barium, the current–voltage relationship shifted into the range of a high-voltage–activated L-type calcium channel (Xu and Lipscombe 2001). Additional factors such as interactions with other subunits, modulation by second-messenger signaling cascades, and alternative splicing have the potential to influence channel properties (Birnbaumer et al. 1998; Lipscombe 2002b; Scholze et al. 2001). However, low-voltage activation appears to be a salient feature of $Ca_V1.3$-containing channels (Koschak et al. 2001; Michna et al. 2003; Safa et al. 2001; Scholze et al. 2001; Xu and Lipscombe 2001).

$Ca_V1.3$ L-type channels are only partially inhibited by dihydropyridines

All L-type calcium channels studied to date are sensitive to dihydropyridine antagonists and agonists. However, $Ca_V1.3$-

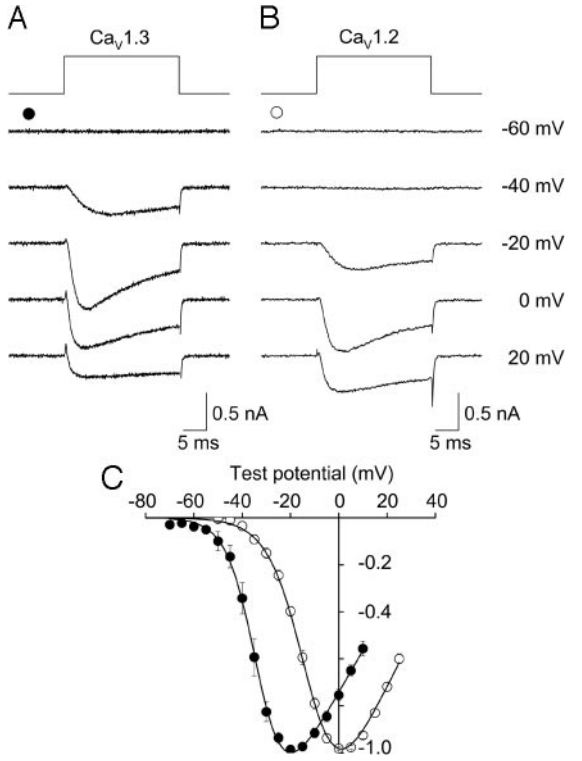

FIG. 2. Ca$_v$1.3 and Ca$_v$1.2 L-type channels have different activation thresholds. Whole cell currents measured from tsA201 cells expressing Ca$_v$1.3 (*A*) and Ca$_v$1.2 (*B*) together with Ca$_V\alpha_2\delta_1$ and Ca$_V\beta_3$. Currents were activated by step depolarizations to the indicated test potentials from a holding potential of −100 mV; 2 mM Ca^{2+} was the charge carrier. *C*: averaged current–voltage relationships for Ca$_v$1.3 (●) and Ca$_v$1.2 (○) channels. Activation $V_{1/2}$ values calculated from Boltzmann-linear fits were, −40.4 ± 0.9 mV and −16.1 ± 0.5 mV for Ca$_v$1.3 and Ca$_v$1.2, respectively (n = 8, 11). For a more detailed description of methods see Xu and Lipscombe (2001). All clones were isolated in our laboratory. Ca$_V$1.3 and Ca$_V\alpha_2\delta_1$ clones were isolated from a rat sympathetic cDNA library, Ca$_V$1.2 from mouse brain, and Ca$_V\beta_3$ from rat brain. Accession numbers are for Ca$_V$1.3: AF370009; Ca$_V$1.2: AY728090; Ca$_V\beta_3$ is identical to M88751; Ca$_V\alpha_2\delta_1$: AF286488.

containing L-type calcium channels appear to be significantly less sensitive to dihydropyridine antagonists (Koschak et al. 2001; Xu and Lipscombe 2001). This property complicates identification of Ca$_V$1.3 currents. For example, >90% of Ca$_V$1.2 current is inhibited by 1 μM nimodipine, but this same concentration inhibits only 50% of peak Ca$_V$1.3 current (Xu and Lipscombe 2001). Striessnig and colleagues obtained similar results using isradipine (Koschak et al. 2001). The lower sensitivity of Ca$_V$1.3 channels to dihydropyridine antagonists becomes even more significant at hyperpolarized membrane potentials. Inhibition by dihydropyridines is state-dependent: enhanced at depolarized membrane potentials that open the channel, but reduced at hyperpolarized membrane potentials (Bean 1984; Berjukow et al. 2000). Consequently, dihydropyridines become particularly ineffective at inhibiting Ca$_V$1.3 currents activated at the foot of the current–voltage curve (Xu and Lipscombe 2001). Interestingly, the Ca$_V$1.3 current that remains in the presence of dihydropyridines takes on the profile of an inactivating current with barium as the charge carrier (Fig. 3) (Xu and Lipscombe 2001). This is consistent with the state-dependent nature of the block by dihydropyridines (Bean 1984; Berjukow and Hering 2001). In their presence, Ca$_V$1.3 channels generate low-threshold, drug-resistant, inactivating currents that resemble the R-type current of many neurons (Foehring et al. 2000; Randall and Tsien 1995; Tottene et al. 1996; Yasuda et al. 2003; Zhuravleva et al. 2001).

Ca$_V$1.3 L-type channels open with rapid kinetics

L-type calcium channels in neurons are typically thought of as slowly activating (Mermelstein et al. 2000; Yasuda et al. 2003). If true, this property limits the involvement of L-type calcium channels to signaling pathways triggered by more prolonged membrane depolarizations. However, Ca$_V$1.2 and Ca$_V$1.3 currents shown in Fig. 2 clearly activate with fast kinetics (Xu and Lipscombe 2001) and, although contrary to the pervading viewpoint, these data are consistent with certain other studies. Cloned Ca$_V$1.2 L-type channels have been shown to support at least as much calcium influx as Ca$_V$2 channels, in response to brief action potential stimuli (Liu et al. 2003) and native L-type channels mediate spike-induced calcium influx in hippocampal dendrites (Christie et al. 1995). It is likely that activation kinetics of L-type calcium channels will vary depending on several factors including cell-type, temperature, alternative splicing, and the presence of auxiliary subunits (Birnbaumer et al. 1998; Lipscombe 2002b; Liu et al. 2003). For example, the Ca$_V$1.2 clone isolated from rabbit heart used in our earlier studies (Xu and Lipscombe 2001) activates with kinetics that are slow as compared with our neuronal Ca$_V$1.2 clone (Fig. 2). However, we also suggest that pharmacological subtraction methods used frequently to isolate L-type calcium channels from other subtypes of voltage-gated calcium channels in neurons, might have contributed to the notion that L-type calcium channels activate with slow kinetics (Fig. 3).

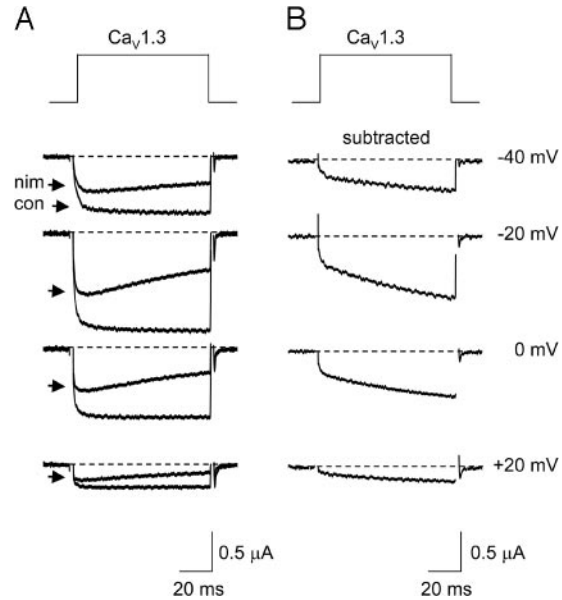

FIG. 3. Dihydropyridine-sensitive component of the Ca$_v$1.3 L-type current appears to activate slowly. *A*: representative Ca$_v$1.3 currents in the absence (black) and presence (gray) of 1 μM nifedipine. Currents were activated by step depolarizations to the indicated potentials from a holding potential of −100 mV. Currents were recorded from *Xenopus* oocytes expressing Ca$_v$1.3, Ca$_v\alpha_2\delta$, and Ca$_v\beta_{1b}$; 5 mM barium is the charge carrier. Additional details can be found in Xu and Lipscombe (2001). *B*: subtracted Ca$_v$1.3 currents showing the dihydropyridine-sensitive component at the indicated test potentials. Currents appear to activate slowing because of the time-dependent nature of dihydropyridine block. Scale bars: 0.5 μA, 20 ms.

The L-type calcium current in neurons is defined frequently as the whole cell calcium current that is inhibited by dihydropyridine antagonists. Pharmacological subtraction is only appropriate, however, when inhibition is complete, and independent of voltage and time; conditions not met for dihydropyridine block of $Ca_V1.3$ L-type currents (Figs. 2, 3). Figure 3 illustrates that $Ca_V1.3$ L-type currents open with rapid kinetics, and inactivate little with barium as the charge carrier. However, in the presence of nimodipine, inhibition is incomplete and the remaining current appears to inactivate. These data are consistent with the state-dependent nature of nimodipine block. Consequently, the dihydropyridine-sensitive, subtracted current appears as slowly activating, not because L-type calcium channels open slowly but because the block is time-dependent and incomplete (Fig. 3).

$Ca_V1.4$ L-type channels are functionally unique

$Ca_V1.4$ was recently cloned from human and mouse retinal tissue and heterologously expressed in mammalian cells (Baumann et al. 2004; Koschak et al. 2003; McRory et al. 2004). Although currents generated from cells expressing $Ca_V1.4$ clones were small, they had properties similar in several respects to those of $Ca_V1.3$. These include rapid activation kinetics, low activation threshold, and lower sensitivity to dihydropyridine inhibition (Baumann et al. 2004; Koschak et al. 2003; McRory et al. 2004). Native L-type currents in retinal cells, presumed to be $Ca_V1.4$-containing (Taylor and Morgans 1998; Wilkinson and Barnes 1996), are likewise similar to recombinant $Ca_V1.3$ and $Ca_V1.4$ channels. They have lower sensitivity to dihydropyridines and activate at negative thresholds. These data are consistent with the relatively high level of sequence homology between $Ca_V1.3$ and $Ca_V1.4$ genes (Fig. 1) (Lipscombe 2002b). Heterologously expressed $Ca_V1.4$ L-type calcium channels are also distinctive in lacking calcium-dependent inactivation (Baumann et al. 2004; Koschak et al. 2003; McRory et al. 2004). The currents in these studies were all relatively small but calcium entering through a single channel should be sufficient to support inactivation, if components of the calcium-dependent inactivation pathway are present (Peterson et al. 2000; Yue et al. 1990). The absence of calcium-dependent inactivation suggests that $Ca_V1.4$ channels are functionally distinct from $Ca_V1.3$ and $Ca_V1.2$ channels (Koschak et al. 2003; McRory et al. 2004).

Native neuronal L-type currents are functionally diverse

Certain cell-types, including hair cells, amacrine cells, and endocrine cells express a limited number of calcium channels. Within this background, low-threshold dihydropyridine-sensitive L-type calcium currents are more readily identified (Ashcroft et al. 1994; Habermann et al. 2003; Liu et al. 2004; Michna et al. 2003; Platzer et al. 2000; Schnee and Ricci 2003; Smith et al. 1993). Several groups also report low-threshold-activating dihydropyridine-sensitive L-type currents in hippocampal pyramidal, cortical striatal, suprachiasmatic, thalamic, and motor neurons with properties similar to $Ca_V1.3$ channels (Avery and Johnston 1996; Cloues and Sather 2003; Li and Bennett 2003; Liljelund et al. 2000; Pennartz et al. 2002; Sand et al. 2001; Svirskis and Hounsgaard 1997; Vergara et al. 2003; Zhuravleva et al. 2001). Notably in 1996, Avery and Johnston commented that "*. . . the designation 'low-voltage-activated' should not be limited to T-type channels*." These investigators "*. . . challenge the traditional designation of L-type channels as exclusively HVA and reveal a possible role in subthreshold Ca^{2+} signaling*" (Avery and Johnston 1996). Single-channel recordings also indicate differences among neuronal L-type calcium channels in hippocampal pyramidal and cerebellar granule cells (Forti and Pietrobon 1993; Kavalali and Plummer 1996; Schjott and Plummer 2000). Recordings distinguish at least 2 gating activities of L-type calcium channels that may represent different channel subtypes. It will be interesting to determine their molecular origins.

Physiological significance

Neuronal L-type calcium channels play established roles in regulating gene expression, cell survival, and synaptic plasticity (Christie et al. 1997; Deisseroth et al. 1998; Galli et al. 1995; Mao et al. 1999; Marshall et al. 2003; Murphy et al. 1991; Norris et al. 1998; Weisskopf et al. 1999). In select cells and synapses, L-type calcium channels can also regulate exocytosis (Ashcroft et al. 1994; Fuchs 1996; Heidelberger and Matthews 1992; Liu et al. 2004; Sand et al. 2001; Thaler et al. 2001; Wiser et al. 1999). In addition, data reviewed here suggest $Ca_V1.3$ L-type calcium channels mediate subthreshold calcium signaling. For example, dihydropyridine antagonists suppress spontaneous intracellular calcium oscillations and slow rhythmic firing in several excitable cells, including cerebellar Purkinje neurons, suprachiasmatic nucleus neurons, inferior olivary neurons, corticostriatial neurons, pituitary cells, and GH3 cells (Charles et al. 1999; Giraldez et al. 2002; Liljelund et al. 2000; Pennartz et al. 2002; Placantonakis and Welsh 2001; Vergara et al. 2003). These studies and those that show sinoatrial node dysfunction in $Ca_V1.3$ knockout mice (Mangoni et al. 2003; Platzer et al. 2000; Zhang et al. 2002) strongly implicate $Ca_V1.3$ L-type calcium channels in driving oscillatory activity. $Ca_V1.3$ channels could also mediate sustained calcium entry during action potential plateaus, as calcium-dependent and voltage-dependent inactivation is minimal at depolarized voltages (Figs. 2 and 3).

Many neurons express low- to mid-threshold activating, inactivating, and drug-resistant calcium currents collectively called R-type. These currents are generally attributed to $Ca_V2.3$ channels (Cloues and Sather 2003; Magistretti et al. 2000; Randall and Tsien 1995; Tottene et al. 1996; Yasuda et al. 2003). The properties of $Ca_V1.3$ channels suggest that distinguishing them from $Ca_V2.3$ channels using dihydropyridine antagonists would be difficult. It is quite likely that $Ca_V1.3$ L-type calcium channels contribute a significant fraction of the R-type current in many neurons. The prevalence of significant drug-resistant R-type currents in neurons of $Ca_V2.3$ knockout mice strongly supports this proposal (Wilson et al. 2000). R-type currents contribute to presynaptic transmitter release at certain synapses (Wu et al. 1999) and to synaptic plasticity in dendritic spines of hippocampal pyramidal neurons (Yasuda et al. 2003).

$Ca_V1.3$ L-type channels will activate in response to physiological stimuli that do not open $Ca_V1.2$ L-type channels. This broadens the functional importance of L-type calcium channels to included neuronal processes triggered by fast, subthreshold depolarizations. Differences in their primary structure and

subcellular distributions also indicate that $Ca_V1.2$ and $Ca_V1.3$ channels are likely to regulate divergent downstream signaling pathways. It will be very interesting to know, for example, if $Ca_V1.3$ channels can couple to transcription factors as efficiently as $Ca_V1.2$ (Dolmetsch et al. 2001; Weick et al. 2003). While we wait for *the low down* on $Ca_V1.3$ channels, a selective inhibitor to differentiate between L-type calcium channel subtypes would be invaluable for establishing their relative contributions to calcium signaling in neurons.

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