Functional Characterization of the L-type Ca\textsuperscript{2+} Channel Ca\textsubscript{v}1.4\alpha1 from Mouse Retina

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PURPOSE. To study the electrophysiological and pharmacological properties of the L-type Ca\textsuperscript{2+} channel (LTCC) Ca\textsubscript{v}1.4\alpha1 (\alpha1F) subunit from mouse retina and assess their contributions to the native retinal channel.

METHODS. The full-length cDNA of Ca\textsubscript{v}1.4\alpha1 was cloned from murine retina in an RT-PCR approach. Ca\textsubscript{v}1.4\alpha1 was expressed alone or together with the auxiliary \alpha2\beta1 and \beta2a or \beta3 subunits in HEK293 cells. The electrophysiological and pharmacological characteristics of L-type Ca\textsuperscript{2+} and Ba\textsuperscript{2+} inward currents (I\textsubscript{Ca} and I\textsubscript{Ba}) induced by Ca\textsubscript{v}1.4\alpha1 were determined by the whole-cell configuration of the patch-clamp method and compared with currents induced by the cardiac and smooth muscle-type Ca\textsubscript{v}1.2\alpha1 (\alpha1C) channel.

RESULTS. Ca\textsubscript{v}1.4\alpha1-mediated I\textsubscript{Ba} was observed only when the \alpha2\beta1 and \beta subunits were coexpressed. Current densities were approximately two times higher with \beta2a than with \beta3. I\textsubscript{Ca} and I\textsubscript{Ba} activated faster and revealed much slower time-dependent inactivation than I\textsubscript{Ca} induced by Ca\textsubscript{v}1.2\alpha1. Unlike in Ca\textsubscript{v}1.2\alpha1, inactivation was not accelerated with Ca\textsuperscript{2+} as the charge carrier, indicating the absence of Ca\textsuperscript{2+}-dependent inactivation in Ca\textsubscript{v}1.4\alpha1. Ca\textsubscript{v}1.4\alpha1 exhibited voltage-dependent inactivation. The dihydropyridine (DHP) antagonist isradipine blocked Ca\textsubscript{v}1.4\alpha1 with approximately 20-fold lower sensitivity than Ca\textsubscript{v}1.2\alpha1. The agonistic DHP BayK 8644 stimulated maximum Ca\textsuperscript{2+} current. Its intrinsic biophysical properties, in particular its unique inactivation properties, enable Ca\textsubscript{v}1.4\alpha1 to provide a sustained I\textsubscript{Ca} over a voltage range such as required for tonic glutamate release at the photoreceptor synapse. (Invest Ophthalmol Vis Sci. 2004;45:708–713) DOI:10.1167/iovs.03-0937

CONCLUSIONS. The data indicate that Ca\textsubscript{v}1.4\alpha1 subunit constitutes the major molecular correlate of retinal L-type Ca\textsuperscript{2+} current. Its intrinsic biophysical properties, in particular its unique inactivation properties, enable Ca\textsubscript{v}1.4\alpha1 to provide a sustained I\textsubscript{Ca} over a voltage range such as required for tonic glutamate release at the photoreceptor synapse.

The release of neurotransmitters from nerve terminals is initiated by Ca\textsuperscript{2+}-influx through presynaptic voltage-dependent Ca\textsuperscript{2+} channels. Recent studies indicate that L-type Ca\textsuperscript{2+} channel (LTCCs) play a key role in the control of tonic glutamate release from retinal photoreceptors and bipolar cells.\textsuperscript{1–3} LTCCs recorded from photoreceptors\textsuperscript{4–7} and bipolar cells\textsuperscript{8–10} possess biophysical and pharmacological properties that set these channels apart from other LTCCs, such as cardiac and smooth muscle LTCCs. These unique properties include fast activation and very slow inactivation kinetics, as well as relatively low sensitivity to DHPs. LTCCs are not restricted to the synaptic layers of the retina. They have also been described in the cell soma of photoreceptors and in somatodendritic localization in bipolar and ganglion cells.\textsuperscript{10–12} These LTCCs are thought to supply the Ca\textsuperscript{2+} entry essential in more general cellular functions, such as Ca\textsuperscript{2+}-mediated signal transduction or the regulation of gene transcription.\textsuperscript{13}

LTCCs are multisubunit proteins consisting of the principal \alpha1 and the auxiliary \beta and \alpha2\beta1-3 subunits. Some LTCCs, such as the skeletal muscle Ca\textsuperscript{2+} channel, contain an additional \gamma subunit.\textsuperscript{14} Whereas \alpha1 subunits determine the principal biophysical and pharmacological properties of the channel, \beta subunits modulate cell surface expression, voltage dependence, and opening kinetics. So far, the functional role of the \alpha2 and the \gamma subunit have been less well investigated.

Three different LTCC \alpha1 subunits have been detected in the neuroretina, Ca\textsubscript{v}1.2\alpha1 (\alpha1C), Ca\textsubscript{v}1.3\alpha1 (\alpha1D), and Ca\textsubscript{v}1.4\alpha1 (\alpha1F).\textsuperscript{12} In contrast to Ca\textsubscript{v}1.2\alpha1 and Ca\textsubscript{v}1.3\alpha1 which are expressed in a variety of tissues, Ca\textsubscript{v}1.4\alpha1 seems to be specifically expressed in the retina.\textsuperscript{16,17} Recently, mutations in the gene encoding Ca\textsubscript{v}1.4\alpha1 (CACNA1F) have been identified in patients who have incomplete X-linked congenital stationary night blindness (CSNB2). The key symptoms of this disease are impaired night vision and decreased visual acuity. The electrophysiological hallmark is the Schubert and Bornschein type electroretinogram, in which the amplitude of the scotopic b-wave is smaller than that of the normal sized a-wave. This finding suggests that the pathologic correlate of the disease is localized most likely at the photoreceptor-to-bipolar synapse.\textsuperscript{20} Although the genetic studies indicate that the functional loss of Ca\textsubscript{v}1.4\alpha1 causes CSNB2, the molecular mechanism by which mutations in this channel lead to disease is not understood. To address this important question in a physiological context, mouse models of this channelopathy are needed. To this end, we set out in this study to clone and functionally express the complete cDNA of Ca\textsubscript{v}1.4\alpha1 from mouse retina. The biophysical and pharmacological properties of Ca\textsubscript{v}1.4\alpha1-mediated calcium currents concurred well with those of calcium currents from photoreceptors and bipolar cells.

MATERIALS AND METHODS

Cloning of Murine Ca\textsubscript{v}1.4\alpha1

Total RNA was purified from retinas of C57Bl/6 mice using the phenol-guanidine-isothiocyanate-chloroform extraction protocol (PeqGOLD Trifast; PeqLab, Erlangen, Germany). All animals were used in compliance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. First-strand cDNA synthesis was performed with 5 µg total RNA, using oligo d(T) primer and reverse transcriptase.

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Table 1. Sequences and Localization of PCR Primers Used for Cloning of Ca_{v1.4}α1

<table>
<thead>
<tr>
<th>Primer Pair</th>
<th>Sequence (5’ → 3’)</th>
<th>Localization of Amplicon (AF192497)</th>
<th>Length of Amplicon (bp)</th>
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<tr>
<td>17</td>
<td>cggatgctggccacactATGGCAAGTCTGAAGTCGGGAA</td>
<td>nt 49-2138</td>
<td>2105</td>
</tr>
<tr>
<td>18</td>
<td>GTATCAAGGGTGCCCTCTTGTTG</td>
<td>nt 2003-4056</td>
<td>2054</td>
</tr>
<tr>
<td>19</td>
<td>CCATGAAATCTCAGGGCTTCTG</td>
<td>nt 5911-6005</td>
<td>2106</td>
</tr>
</tbody>
</table>

Coding sequences are represented in uppercase letters. 5’- and 3’-untranslated sequences are shown in lowercase letters.

(Superscript II; Invitrogen, Karlsruhe, Germany). To obtain the complete coding region of Ca_{v1.4}α1, three overlapping PCR fragments were amplified from the cDNA using the Expand long template PCR system (Roche Diagnostics, Mannheim, Germany). The primers were designed according to the murine Ca_{v1.4}α1 sequence AF192497 (Table 1). The PCR fragments were subcloned into pUC18 or pcDNA3 (Invitrogen, Karlsruhe, Germany) and several clones for each fragment were sequenced on both strands using the a chain terminator cycle sequencing kit (BigDye Terminator; Applied Biosystems, Foster City, CA). A eukaryotic expression vector for Ca_{v1.4}α1 was constructed by ligating the 5945-bp EcoRI/BamHI fragment (nucleotides [nt] 48-3992) containing an optimized sequence for initiation of translation and the 2013-bp BamHI/XhoI fragment (nt 3993-6005) into an EcoRI/SalI-cut bicistronic pIR/RES2-EGFP vector (Clontech, Heidelberg, Germany).

Heterologous Expression in HEK293 Cells and Electrophysiological Analysis of Ca_{v1.4}α1

HEK293 cells were transiently transfected with expression vectors encoding Ca_{v1.4}α1 or the Ca_{v1.2}α1, respectively, together with equimolar amounts of vectors encoding β2x^{32} or β2^{32} and α2B^{21,22} using transfection reagent (Fugene 6; Roche Diagnostics). For control experiments HEK293 cells were transfected with the empty enhanced fluorescent protein (EGFP) vector. Cells were cultured in DMEM supplemented with 10% fetal calf serum and kept at 37°C, 10% CO_{2}, I_{ch} and I_{rest} was measured from EGFP-positive cells using the following solutions (mM): (pipette solution) 112 CsCl, 3 MgCl_{2}, 3 MgATP, 10 EGTA, and 5 HEPES, adjusted to pH 7.4 with CsOH; (bath solution) 82 NaCl, 30 BaCl_{2}, 5.4 CsCl_{2}, 1 MgCl_{2}, 20 tetraethylammonium (TEA), and 5 HEPES, and 10 glucose, adjusted to pH 7.4 with NaOH. For experiments with 10 mM Ba^{2+} or 10 mM Ca^{2+} in the bath solution, the NaCl concentration was increased to 102 mM.

Currents were recorded at room temperature 2 to 4 days after transfection, using the whole-cell patch-clamp technique. Data were acquired at 10 kHz with an amplifier (Axopatch 200B with pClamp 8; Axon Instruments, Foster City, CA). Voltage-clamp data were stored on the computer hard disc and analyzed off-line (Clampfit 8; Axon Instruments, and Origin 6.1; OriginLab Co., Northampton, MA).

Patch pipettes were pulled from borosilicate glass and when filled with pipette solution, their input resistance was between 1.2 and 2.0 MΩ. Typical cell sizes were between 15 and 60 pF. Access resistances were between 3.0 and 4.0 MΩ and were compensated up to 70%. The holding potential was −80 mV, unless stated otherwise.

The peak I-V relationship was measured by applying 150-ms voltage pulses to potentials between −80 and +70 mV in 10 mV increments from a holding potential of −80 mV at 0.2 Hz. To obtain current densities, the current amplitude at V_{max} was normalized to cell membrane capacitance (C_{m}). From I-V curves the activation threshold was determined as the test potential at which 5% of the maximum current was activated.

For determination of half-maximum activation voltage (V_{0.5,act}) the chord conductance (G) was calculated from the current voltage curves by dividing the peak current amplitude by its driving force at that respective potential G = I/(V - V_{rev}), where V_{rev} is the extrapolated reversal potential, V is the membrane potential, and I is the peak current. The chord conductance was then fitted with a Boltzmann equation G = G_{max}/(1 + e^{(V_{0.5,act} - V)/k}), where G_{max} is the maximum conductance, V_{0.5,act} is the half-maximum activation voltage, V is the test potential, and k is the slope factor of the activation curve.

For determination of half-maximum inactivation voltage (V_{0.5,inact}) steady state inactivation curves were measured from a holding potential of −80 mV, by using a series of conditioning pre-pulses of different length to various voltages between −100 mV and +50 mV. For Ca_{v1.2}α1, the duration of the conditioning prepulse was 5 seconds, for Ca_{v1.4}α1 pulses of 5, 10, 20, and 30 seconds were applied to achieve steady state. The conditioning pulse was followed by a 20-ms return to the holding potential and a 500-ms test pulse to V_{max} at 0.1 Hz or 0.05 Hz. Tail currents immediately after the final step to V_{max} were normalized to maximum current amplitude and plotted as a function of the preceding membrane potential. The data points were fitted with the Boltzmann function: I = I_{0} + I_{max}/[1 + e^{(V_{0.5,inact} - V)/k}], where I_{0} is the test potential, V_{0.5,inact} is the half-maximum voltage for steady state inactivation, and k is the slope factor of the curve.

The time course of Ca_{v1.2}α1 current activation was fitted by the monoequponential function: I_{A} = A_{0} · e^{-t/\tau_{A}} + C, where I_{A} is the current at time t after a voltage pulse to V_{max}, A_{0} is the steady state current amplitude with the respective time constant of activation, \tau, and C is the remaining steady state current. Ca_{v1.4}α1 current activation was fitted by the biexponential function: I_{A} = A_{fast} · e^{-t/\tau_{fast}} + A_{slow} · e^{-t/\tau_{slow}} + C, where \tau_{slow} and \tau_{fast} represent slow and fast time constants of activation, respectively.

To characterize the pharmacological properties of Ca_{v1.4}α1, we tested LTCC antagonists and the agonist DHP Bayk 8644. Drugs were applied by a local solution exchanger and reached the cell membrane within less than 100 ms. The effects of the antagonists were tested with 40-ms voltage-clamp steps to 0 mV or +10 mV from HPs of −80 mV or −50 mV. Pulse frequency was 0.2 Hz. For each test configuration, drug effects were measured after steady state block was attained within 2 to 3 minutes after drug application. For each antagonist the ratio I_{drug}/I_{control} was calculated from the peak current-voltage relations. The concentration-inhibition curve derived from responses to four to five different concentrations was obtained by fitting I_{drug}/I_{control} to the Hill equation 1/[1 + (IC_{50}/G)]^{n}, where G is the drug concentration, n_{H} is the Hill coefficient, and IC_{50} is the drug concentration needed for half-maximum block.

Stock solutions of drugs were prepared in H_{2}O or ethanol (isradipine) and stored at −4°C in the dark. For electrophysiological measurements, stock solutions were freshly diluted in bath solution. Racemic verapamil HCl, d-cis-diltiazem, and l-cis-diltiazem were purchased from Sigma-Aldrich Corp. (St. Louis, MO), d-cis-diltiazem was purchased from Biomol Research Laboratories Inc. (Plymouth Meeting, PA), and racemic isradipine was a gift from Novartis Pharma AG (Basel, Switzerland).

Statistics

All results are expressed as the mean ± SEM; n is the number of experiments. An unpaired t-test was performed for the comparison between two groups. Significance was also tested by ANOVA, if multiple comparisons were made. P < 0.05 was considered significant.
30 mM Ba\(^{2+}\) was not different from control cells (Fig. 1B). The threshold for Ca\(^{2+}\) activation current was \(-28 \pm 1.2\) mV (n = 30), which is 5 mV more negative than Ca\(^{2+}\) current activation \((-23.4 \pm 1.9\) mV; n = 9). For both \(\alpha_1\) subunits, the peak current occurred at similar \(V_{\text{max}}\) with 13.8 \pm 0.9 mV (n = 30) for Ca\(^{2+}\) and 13.5 \pm 1.7 mV (n = 9) for Ca\(^{2+}\); (Fig. 1B). The peak current densities for Ca\(^{2+}\) was \(-9.5 \pm 1.1\) pA/pF (n = 30) and \(-31.4 \pm 9.4\) pA/pF (n = 9) for Ca\(^{2+}\). To compare the voltage-dependent activation and inactivation of the Ca\(^{2+}\) and Ca\(^{2+}\) currents, normalized conductance-voltage relations and steady state inactivation curves of \(I_{\text{Na}}\) were determined and fitted by Boltzmann distributions (Fig. 1C). The activation curves for both \(\alpha_1\) subunits were almost identical. The potential of half-maximum \(I_{\text{Na}}\) activation \((V_{\text{Na}_{1/2}})\) was \(1.1 \pm 0.0\) mV for Ca\(^{2+}\) (n = 28) and \(-0.1 \pm 1.2\) mV for Ca\(^{2+}\) (n = 9). In contrast, at a conditioning pulse duration of 5 seconds, the steady state inactivation curve of Ca\(^{2+}\) was shifted to approximately 20 mV more depolarized potentials with respect to Ca\(^{2+}\). The potential of half-maximum \(I_{\text{Na}}\) inactivation \((V_{\text{Na}_{1/2}})\) was \(0.64 \pm 2.6\) mV for Ca\(^{2+}\) subunit (n = 9) and \(-24.3 \pm 1.5\) mV for Ca\(^{2+}\) subunit (n = 9).

Voltage-dependent inactivation of Ca\(^{2+}\) was dependent on the conditioning pulse prepulse. Increasing the pulse duration from 5 to 30 seconds increased \(I_{\text{Na}}\) inactivation and shifted \(V_{\text{Na}_{1/2}}\) to approximately 25 mV more hyperpolarized voltages (Fig. 2). There was no significant difference between the inactivation curves for the 20-second and 30-second pulse duration, indicating that steady state was achieved after 20 seconds.

Ca\(^{2+}\) also formed functional channels with the \(\beta_3\) subunit, an auxiliary LTCC subunit that is expressed in the central nervous system (CNS) and in a variety of peripheral tissues. Peak current densities obtained after coexpression of Ca\(^{2+}\) and \(\beta_3 + \alpha_2\) were less than half those recorded in the presence of \(\beta_2a\) (\(\beta_3: -4.4 \pm 0.8\) pA/pF, n = 10; \(\beta_2a: -9.5 \pm 1.1\) pA/pF, n = 30). However, the biophysical properties of both currents were very similar to each (Table 2). As the only exception, the activation threshold was slightly shifted to more hyperpolarized voltages in the presence of \(\beta_2a\) (\(\beta_3: -28.0 \pm 1.2\) mV, n = 30; \(\beta_3: -37.2 \pm 2.4\) mV, n = 10). Ca\(^{2+}\) is not only the permeating cation of LTCCs, it is also a key determinant of their inactivation. Figure 3A shows current traces of Ca\(^{2+}\) in comparison to current traces of the smooth muscle and cardiac type Ca\(^{2+}\). It is evident that Ca\(^{2+}\) activated faster \((\tau_{\text{fast}}:\ 0.65 \pm 0.04\) ms; \(\tau_{\text{slow}}: 3.6 \pm 0.58\) ms; n = 24; relative contribution of slow component: 0.35 \pm 0.05) than Ca\(^{2+}\) (\(\tau: 1.59 \pm 0.47\) ms, n = 8). Moreover, Ca\(^{2+}\) displayed extremely slow inactivation kinetics. During the 150-msec voltage step shown in Figure 1A the current did not significantly decrease. At +10 mV it took more than 30 seconds for full inactivation (not shown). Consistent with the properties of an LTCC, Ca\(^{2+}\) activated at relatively positive membrane potentials. In experiments performed with 30 mM Ba\(^{2+}\) as the charge carrier, the mean I-V relationship for Ca\(^{2+}\) and Ca\(^{2+}\) were almost identical (Fig. 1B). For all experiments Ca\(^{2+}\) or Ca\(^{2+}\) was coexpressed with \(\alpha_2\) and \(\beta_2\). Currents were measured in bath solution containing 30 mM Ba\(^{2+}\) as the charge carrier. Biophysical parameters are given in Table 2. Whole-cell current recorded from representative cells expressing either Ca\(^{2+}\) (left) or Ca\(^{2+}\) (right). Currents were recorded from a HP of \(-80\) mV by applying 150-msec pulses to membrane voltages between \(-80\) mV and +70 mV at 0.2 Hz. (B) I-V relationship for Ca\(^{2+}\) channels (■ n = 50) and Ca\(^{2+}\) (○ n = 9). Individual I-V curves were normalized to the respective maximum current and then averaged. (C) Conductance-voltage relationships for Ca\(^{2+}\) (■ n = 28) and Ca\(^{2+}\) (○ n = 9) and steady state inactivation curves for Ca\(^{2+}\) (■ n = 9) and Ca\(^{2+}\) (○ n = 9) were determined. Individual curves were normalized to maximum current amplitude and then averaged. Solid lines: fits of the data to the Boltzmann equation. Inactivation curves were determined using a conditioning prepulse of 5 seconds and a 300-ms test pulse to +10 mV.

**RESULTS**

**Molecular Cloning of Murine Ca\(^{2+}\)**

To clone murine Ca\(^{2+}\) we designed specific primer pairs based on the previously published sequence of this channel (Table 1) and performed RT-PCR with retinal cDNA from mouse strain C57BL6. The full-length cDNA of Ca\(^{2+}\) was determined to be 6111 bp with an open reading frame encoding a protein of 1984 amino acid residues (GenBank accession number of murine Ca\(^{2+}\): AJ759852; available by ftp at zippy.nimh.nih.gov/ or at http://rsb.info.nih.gov/nih-image; developed by Wayne Rasband, National Institutes of Health, Bethesda, MD). The sequence is nearly identical with the mouse sequence published by Naylor et al. with the notable exception of a strongly diverging sequence stretch in the C terminus (amino acid residues 1768-1807). Inspection of the nucleotide sequences revealed that the profound differences in this stretch arise from frame shifts in the cDNA. The strong homology between the sequence published in this study to that of rat and human Ca\(^{2+}\) (see sequence alignment in Supplemental Figure 1, available online at http://www.iovs.org/cgi/content/full/45/2/708/DC1) strongly indicates that our sequence version represents the correct one.

**Electrophysiological Properties of Murine Ca\(^{2+}\)**

HEK293 cells transfected with Cav1.4 were transfected with an empty vector (current densities at \(V_{\text{max}}\) in
Cav1.4

TABLE 2.

IC50 of 200 nM isradipine blocked 41.9% of Ca2+ at 80 mV. 10 mM Ca2+ increased the block to 88.3%

Pharmacological Characterization of Ca v1.4

To characterize the pharmacological profile of Ca v1.4α1 we quantified the blocking effect of the Ca2+ channel antagonists isradipine, verapamil, L-cis-diltiazem, and D-cis-diltiazem on I f. At −80 mV the DHP antagonist isradipine blocked I f elicited by depolarizing pulses to V max given at 0.2 Hz with an apparent IC50 of 200 ± 50 nM (n = 5–9; Fig. 4A). At a holding potential of −80 mV 100 nM isradipine blocked 41.9% ± 0.05% (n = 7) of I f. Changing the holding potential to −50 mV significantly increased the block to 88.3% ± 0.01% (n = 9) of I f, indicating a strong voltage-dependence of the observed block (Fig. 4A). Cav1.4α1 was only weakly sensitive to verapamil. At an HP of −80 mV 100 µM verapamil blocked 69.1% ± 0.13% (n = 5) of I f.

We also tested the blocking effect of the two enantiomers of diltiazem under identical conditions. Unexpectedly, the concentration-response relationships were not statistically different from each other (Fig. 4A). L-cis-Diltiazem blocked I f with an IC50 of 74.8 ± 8.3 µM (n = 4–6), and D-cis-diltiazem blocked I f with an IC50 of 91.6 ± 9.4 µM (n = 4–6). This observation is very surprising, because the affinity of L-cis-diltiazem for LTCCs is usually several orders of magnitude lower than that of D-cis-diltiazem.25

Finally, we tested the effect of the DHP agonist BayK 8644 on Cav1.4α1. At a concentration of 1 µM this substance increased the current density of I f approximately sixfold. As in other LTCCs, BayK 8644 shifted the I–V relationship to approximately 8 to 10 mV more hyperpolarized potentials (Fig. 4B, Table 2).

DISCUSSION

In this study we report the cloning and the functional characterization of the Cav1.4α1 calcium channel from mouse retina. Cav1.4α1 requires the coexpression of auxiliary β and α2δ subunits to yield calcium currents in HEK293 cells. This finding indicates that Cav1.4α1, like other members of the LTCC family forms a functional multisubunit complex in the plasma membrane. Currents obtained on coexpression with β2 had consistently bigger amplitudes than currents obtained with β3. One explanation for this finding is that although Cav1.4α1 principally can assemble with different β subunits, it preferentially binds to β2. The key role of the β2 subunit is also demonstrated by a recent study showing that elimination of this subunit in mouse retina produces a phenotype similar to...
For all experiments Ca v1.4 was coexpressed with α2β5, and β2a. Currents were measured in bath solution containing 30 mM BaCl2 as the charge carrier. (A) Concentration-response curves for inhibition of Ca v1.4α1 by cis- and trans-diltiazem (v; n = 5–6), cis- and trans-diltiazem (w; n = 5–6), and isradipine (●; n = 5–9). Pronounced voltage dependence of isradipine block was observed when HP was changed from −80 mV to −50 mV (v; n = 9). (B) I–V relationship for Ca v1.4α1 in the absence (●; n = 50) and presence of 1 μM Bay K 8644 (w; n = 5).

CSNB2 in humans.26 In contrast, no retinal phenotype has been reported so far for β3-27 and β4-deficient mice.28 Taken together, these findings strongly indicate that the β2 subunit is obligatorily required for the formation of native retinal LTCCs.

Ca v1.4α1 possesses unique biophysical and pharmacological properties that set this channel apart from the cardiac and smooth muscle Ca v1.2α1. Currents induced by Ca v1.4α1 activate with very fast kinetics, but display an extremely slow time course of inactivation. At Vm, inactivation of Iinact requires many seconds. Inactivation of Ca v1.4α1 is not accelerated in the presence of extracellular Ca2+. Typical LTCCs such as Ca v1.2α1 are strongly regulated by the passage of Ca2+ through the channel pore. Entering Ca2+ interacts with calmodulin bound to the carboxyl terminus of the channel, thereby causing a decay of the current within milliseconds.14,24 The lack of Ca2+-dependent inactivation in Ca v1.4α1 indicates that either the binding of calmodulin itself or the conformational steps coupling Ca2+-binding to channel inactivation are impaired in this channel. The C-terminal sequences conferring Ca2+-dependent inactivation in Ca v1.2α1 are highly conserved in Ca v1.4α1. It is not known whether the few amino acid exchanges in these sequences explain the different inactivation properties of both channels. Alternatively, not yet identified channel domains may cause the lack of Ca2+-dependent inactivation in Ca v1.4α1. In the absence of the Ca2+-dependent mechanism the time course of currents through Ca v1.4α1 is determined primarily by voltage-dependent inactivation, causing the extremely slow current decay typical of Ca v1.4α1.

Ca v1.4α1 channels reveal a unique pharmacological profile. As for native retinal LTCCs, the apparent DHP antagonist sensitivity of Ca v1.4α1 is approximately 20-fold lower than for the cardiac and smooth muscle Ca v1.2α1 at −80 mV. Twelve of 13 amino acid residues required for high DHP sensitivity in Ca v1.2α1 are present in the primary sequence of Ca v1.4α1.26 As the only difference, Ca v1.4α1 contains a phenylalanine at position 1414 instead of the tyrosine found in Ca v1.2α1. However, this exchange cannot account for the low affinity because Ca v1.4α1, a channel with a DHP sensitivity similar to that of Ca v1.3α1,25 contains at the equivalent position a tyrosine, as does Ca v1.2α1. Although it cannot be excluded that there are other amino acids not yet identified that determine apparent affinities, it is probable that the differences reflect the profound voltage-dependence of the DHP block. In contrast to Ca v1.2α1, Ca v1.4α1 inactivates very slowly at negative holding potentials. Because DHPs bind preferentially to the inactivated state of LTCCs a decrease of apparent affinity is caused by this behavior. Indeed, shifting the holding potential from −80 to −50 mV, hence increasing the fraction of channels being in the inactivated state, strongly increased the DHP block of Iinact. The specific inactivation properties could also underlie the relatively low sensitivities of Ca v1.4α1 currents to verapamil and diltiazem. Unexpectedly, l-cis-diltiazem and l-trans-diltiazem blocked Iinact through Ca v1.4α1 with almost the same apparent IC50. In other LTCCs the affinity of l-trans-diltiazem is at least 20 times higher than that of l-cis-diltiazem.25,38,39 In Ca v1.2α1 the binding site for diltiazem is overlapping with that for DHPs.40 With the exception of the Y1414F exchange the identified amino acids are completely represented in Ca v1.4α1, indicating that other channel domains and/or a differential voltage dependence of the block by the D and L enantiomer of diltiazem determine the observed difference. Micromolar concentrations of l-cis-diltiazem are commonly used to block Ca2+ flux through cyclic nucleotide-gated (CNG) channels in rod and cone photoreceptors.41,42 Because comparable concentrations also block calcium currents through Ca v1.4α1, extreme care is necessary to distinguish the effects of this blocker on CNG channels from those on retinal LTCCs.

In summary, currents through heterologously expressed Ca v1.4α1/B2aα2δ1 closely resemble the slowly inactivating Ca2+ and Ba2+ currents observed in native photoreceptors and bipolar cells.8,9 As the only difference, Ca2+ currents from native photoreceptors and bipolar cells activate at 10 to 15 mV more negative potentials4–6,9,10 than currents obtained after heterologous expression of Ca v1.4α1. Several factors, such as the absence of auxiliary channel subunits, cytosolic modulators or posttranslational modifications in HEK293 cell lines, may explain the discrepancy between native and expressed channels. Nevertheless, its unique electrophysiological properties predestine Ca v1.4α1 to fulfill the specific tasks required for normal retinal function. In particular, the slow inactivation of Ca v1.4α1 is well suited to mediate tonic glutamate release from synaptic terminals of ribbon synapses in photoreceptors and bipolar cells.3 The properties of mouse Ca v1.4α1 are consistent with those of the heterologously expressed human Ca v1.4α1.43 For example, both channels lack Ca2+-dependent inactivation, assemble with β-subunits and reveal intermediate DHP sensitivity. The human Ca v1.4α1 activates at a slightly more negative voltage than the murine Ca v1.4α1. The small difference may be attributed to an intrinsic species difference between mouse and humans or, alternatively, may be due to the slightly different experimental setup used to study both currents. Taken together, our results indicate that mice deficient in this channel will be a useful in vivo model for studying CSNB2.


