Research Paper

The Ca_{1.4} Calcium Channel

More than Meets the Eye

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ABSTRACT

Ca_{1.4} channels are the latest calcium channels to be described in the literature. Originally identified in 1997 from the human genome project, several reports have since been published describing mutations in the CACNA1F gene encoding Ca_{1.4} channels, and implicated these mutations in human disorders such as X-linked cone rod dystrophy (CORDX3) and incomplete X-linked congenital stationary night blindness type 2 (CSNB2). The gene was subsequently cloned and expressed in heterologous expression systems beginning in 2003, and many of the mutations linked to CSNB2 have been tested. Here, we review literature describing the discovery of the CACNA1F gene, its tissue expression profile, alternative splicing events, and biophysical and pharmacological characteristics of the channel in various expression systems. Channel biophysics are also compared to those obtained from recordings made from vertebrate photoreceptors, suggesting that these studies may have been describing Ca_{1.4} channels in native cells.

INTRODUCTION

Influx of calcium ions into cells leads to numerous intracellular events. In many cells, rises in intracellular calcium lead to activation of calcium-dependent signalling cascades and gene activation through signalling to the nucleus. These calcium-mediated events include muscle contraction, neurotransmitter release, insulin release from pancreatic beta cells, activation of immune cells, and programmed cell death. Therefore, intracellular calcium concentrations must be highly regulated.

Voltage-gated calcium channels are integral membrane proteins which gate in response to changes in membrane voltage to permit calcium ions to flow down the electrochemical gradient from the extracellular milieu into the cytoplasm. They are typically comprised of a pore-forming \( \alpha \) subunit, and may associate with other ancillary subunits in vivo, which help to target the channel to the extracellular membrane and alter the biophysical and pharmacological profiles of the channel. The \( \alpha \) subunit shares a topology that is analogous to that of voltage-gated sodium channels whereby a single gene encodes four homologous domains (reviewed in ref. 1). Each domain (I–IV, Fig. 1) consists of six membrane-spanning helical segments (S1–S6), with the fourth segment containing positively charged amino acids at every third position to form the voltage-sensor of the channel. The linker between S5 and S6 forms a re-entrant loop and is thought to line the pore, just as in potassium channels.\textsuperscript{1} Each of the four re-entrant loops contains a critical glutamate residue which pack together to form a ring of negative charge, forming the selectivity filter that permits the passage of divalent ions over monovalent ions.\textsuperscript{2} While the pore-forming \( \alpha \) subunit may be sufficient to form a functional channel, auxiliary \( \alpha_{2}\delta, \beta \) and \( \gamma \) subunits may associate with the channel and alter is functional properties.\textsuperscript{3}

Since calcium influx is critical, several different calcium channels have evolved, each differing in biophysical and pharmacological properties, to permit calcium influx under varying conditions in different cells. Ten genes encoding \( \alpha \) subunits have been identified,\textsuperscript{4,5} and the proteins they encode are broadly classified as either low- or high-voltage activated calcium channels. Ca_{3.1}/\( \alpha_{1C} \), Ca_{3.2}/\( \alpha_{1H} \) and Ca_{3.3}/\( \alpha_{1I} \) are members of the low-voltage activated class as they typically activate near resting membrane potentials, and comprise the T-type calcium channel family. Two families, Cav1 and Cav2, comprise the high-activated calcium channels, requiring relatively larger membrane depolarizations to activate, and include L-type (Ca_{1.1}/\( \alpha_{1A} \), Ca_{1.2}/\( \alpha_{1D} \), Ca_{1.3}/\( \alpha_{1F} \), Ca_{1.4}/\( \alpha_{1G} \)), P/Q-type (Ca_{2.1}/\( \alpha_{1A} \), N-type (Ca_{2.2}/\( \alpha_{1B} \)) and R-type (Ca_{2.3}/\( \alpha_{1C} \)) channels.\textsuperscript{5} Here, we review literature concerning Ca_{1.4} channels, beginning with identification of the gene.
In 1997, the Xp11.23-p11.22 localization of the Ca channel have shown multiple calcium channel subtypes expressed throughout although the presence in the outer plexiform layer was not found in outer nuclear, inner nuclear, and ganglion cell layers of the retina, revealed the presence of Ca was originally reported as retinal specific. A limited number of tissue samples expression of Ca led to the discovery of the full length gene, although it was several years before the cDNA was cloned and expressed (see below). Leads to the discovery of the full length gene, although it was several years before the cDNA was cloned and expressed (see below).

**CHARACTERIZATION OF THE CACNA1F GENE**

Identification of CACNA1F. In 1997, the Xp11.23-p11.22 interval of the X chromosome was of particular interest as it was implicated in numerous X-linked inherited conditions such as Wiskott-Aldrich syndrome, retinitis pigmentosa 2, three forms of X-linked hypercalcemic nephrolithiasis, congenital stationary night blindness, and Aland Island eye disease.

Extensive work from the early stages of the human genome project produced sequence data, where Fisher et al. sequenced over 40 000 bases and showed four genes in close proximity: A4, LM06 (a gene related to mouse testis), synaptophasin, and 16 exons corresponding to the 3’ distal end of the CACNA1F gene. These 16 exons encoded an RNA of 1335 nucleotides in length, and predicted a translated protein of 445 amino acids with high homology to L-type calcium channels. Initial Northern blot analysis with a 445 base pair probe showed high mRNA expression in skeletal muscle and lack of expression in brain or heart.

Subsequent linkage analysis on patients with incomplete X-linked congenital stationary night blindness type 2 (CSNB2) mapped to a similar region of the X chromosome, leading to the hypothesis that mutations in CACNA1F are responsible for the CSNB2/CORDX3 phenotypes. Sequencing of overlapping cosmid (genomic) clones led to the discovery of the full length gene, although it was several years before the cDNA was cloned and expressed (see below).

**Tissue expression.** Using either Northern or RT-PCR analysis on a limited number of tissue samples expression of Ca mRNA was originally reported as retinal specific. In situ hybridization revealed the presence of Ca transcript in the outer plexiform, outer nuclear, inner nuclear, and ganglion cell layers of the retina, although the presence in the outer plexiform layer was not found in other studies.

Immunohistochemical experiments using different antibodies have shown multiple calcium channel subtypes expressed throughout the retina. However, experiments designed to determine the localization of the Ca channel have led to varying results. Using a pan-α channel antibody, Morgans found localized expression of high voltage-activated calcium channels in the outer plexiform layer in rat retinal sections, as well as labelling in the inner segments, inner plexiform, and ganglion cell layers. Using a specific anti-Ca channel directed towards rat sequences gives staining in the outer nuclear layer but not in the outer plexiform layer. A mouse monoclonal antibody gives distinct labelling in the outer plexiform layer and staining in the ganglion cell layer and inner segments, similar to results obtained with a human Ca channel in human retinal sections.

Analysis of expressed sequence tags (ESTs) using the unigene system within the NCBI database reveals that Ca mRNA is transcribed in numerous other tissues in addition to the retina. For example, screening the EST database with the entire Ca mRNA in a nonredundant manner reveals the presence of Ca ESTs in eye, lung, heart, skeletal muscle, thymus, cranial (optic) nerve, lymphocytes, and endocrine tissue including the pineal gland (see Table 1). Ca mRNA has been detected in skeletal muscle and pineal gland, as well as adrenal gland, bone marrow, spinal cord, spleen, and thymus. In addition, Western blot analysis with specific antibodies has detected Ca protein in spleen, spinal cord, bone marrow, and thymus, in addition to retina. Several reports have also recently described the presence of Ca mRNA in T lymphocytes. Thus, Ca appears to be distributed throughout various tissues, suggesting a broader role in physiology than first implicated. However, one caveat we note is that if Ca mRNA or protein is expressed in lymphocytes, then the migrating pattern and accumulation of lymphocytes in different tissues could give false positives in these tissues if lymphocytes were present at the time the tissue libraries/mRNA were prepared. Immunocytohistochemistry using specific Ca antibodies needs to be performed on these tissues in order to verify that the protein is indeed expressed, and not the result of a signal due to the presence of T lymphocytes.

**Alternative splicing.** Voltage-gated calcium channel genes typically contain numerous (up to 48) exons and introns, allowing for transcription of multiple variant mRNAs by alternative splicing during RNA processing. The Ca channel is highly susceptible to alternative splicing and numerous productive and non-productive mRNA transcripts have been isolated. For example, Boycott et al. have demonstrated the first 3 exons of murine Ca mRNA can be spliced in numerous different

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**Table 1** Summary table of EST database search for Ca ESTs

| Accession # | Tissue Accession # | Tissue |
|-------------|--------------------|--------|
| BX099776.1  | Eye                | BM728193 | Eye |
| CD369408.1  | Lung (alveolar macrophage) | AL712794 | Skeletal muscle |
| CD369391.1  | Lung                | BQ186213 | Fetal retina |
| BX642510    | Muscle              | BU619350 | Chondrosarcoma |
| CK299179    | Eye                 | BQ184745 | Eye |
| CK301055    | Eye                 | AA019974 | Eye |
| BF847103    | Lung                | BQ636841 | Eye |
| DB119441    | Thymus              | CB757507 | Pineal gland |
| BM706930    | Cranial nerve #2 (optic) | CB807163 | Pineal gland |
| BM703915    | Eye                 | CB811292 | Pineal gland |

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**Figure 1.** Schematic representation of a voltage-gated calcium channel pore-forming α1 subunit. Each gene encodes the protein which consists of four homologous domains (I–IV). Each domain is comprised of six transmembrane helices. Note that both the N- and C-termini are intracellular. The II linker is the site of the auxiliary β subunit binding, and regions implicated in calmodulin-dependant CDI are in the C-terminus. Mutations implicated in CSNB2 and CORDX3 are distributed throughout the protein and not shown.
combinations. Alternative splicing of exon 9 occurs in a region which is in close proximity to the auxiliary beta subunit interaction site located in the I-II linker of the channel (Fig. 1). Splicing in this region may alter interactions of the channel with the auxiliary beta subunit, although to date there have been no studies investigating this. Additionally, exon 32 reported by Bech-Hansen et al. has yet to be identified in cDNA libraries, suggesting that this exon may be extremely rare or a misread from the GENSCAN analysis and is actually intronic sequence.

Recently, mRNA isolated from T lymphocytes demonstrates skipping of 4 exons (31-34) which encode for most of domain IVS3-IVS5, deleting a portion of the reading frame for domain IV. The authors proposed that removal of the voltage sensor in domain IV could render the channel insensitive to membrane depolarizations and, similar to Ca_{1.1} calcium channel in skeletal muscle, link the calcium channel to intracellular calcium stores. However, the Ca_{1.4} alternatively spliced T lymphocyte mRNAs were not expressed to demonstrate a functional channel and the biophysical properties of this novel Ca_{1.4} splice variant remain unknown if it is expressed at all as a functional protein. It is also unknown if this alternative splicing event is limited to T lymphocytes and whether or not it results in distinct gating mechanism unique to these cells.

**BIOPHYSICAL AND PHARMACOLOGICAL CHARACTERIZATION OF TRANSIENTLY EXPRESSED Ca_{1.4}**

**Ca_{1.4} activation.** To facilitate biophysical characterization of the channel in the absence of other contaminating voltage-gated calcium channel currents, several groups have characterized transiently expressed Ca_{1.4} channels in various expression systems when coexpressed with auxiliary alpha and beta subunits. Koschak et al. described human Ca_{1.4} channels, transiently expressed in tsA-201 cells using whole-cell patch clamp techniques and 15 mM Ba^{2+} charge carrier; the authors reported small currents that begin to activate around -40 mV, peak at -6 mV, and display a half-activation potentials (V_{1/2}) of -10 mV. Activation is more negative than those found for Ca_{1.2} and more positive than those found for Ca_{1.3} recorded under identical conditions. Subsequent reports confirmed these results for human Ca_{1.4} expressed in tsA-201 or Xenopus laevis, and murine Ca_{1.4} in HEK293 cells. Interestingly, significant Ca_{1.4} currents above endogenous currents are observed in only a fraction of transfected cells (22% with Ca_{1.4}, compared to 66% for Ca_{1.3} and >90% for Ca_{1.2} under identical conditions). In summary, transiently transfected Ca_{1.4} currents, when recorded in barium, begin to activate near -40 mV a voltage close to the resting membrane potential of photoreceptors.

Single channel analysis of human Ca_{1.4} channels transiently expressed in tsA-201 cells reveals unique features of gating: 1) Ca_{1.4} single channel conductance is approximately five-fold smaller than other Ca_{1} channels (for example, Ca_{1.2} recorded under identical conditions) 2) open probability of Ca_{1.4} is significantly smaller than Ca_{1.2} at all test potentials examined 3) Ca_{1.4} channels have significantly higher fraction of blank sweeps than Ca_{1.2} channels and 4) Ca_{1.4} channels display only a fast open time (mode 1 gating) and do not display the additional longer open time (mode 2 gating) found in Ca_{1.2}. Taken together, these data could explain why, despite abundant channel protein in the membrane, the percentage of transfected cells with observable current is so small. The single channel conductance measured for Ca_{1.4} channels in the expression system differs markedly from single channel conductances observed in rod photoreceptor cells (reviewed in ref. 36). This difference may be explained if rod photoreceptors were to express an unknown protein, which increases or decreases the single channel conductance, or if the transiently expressed channels only display a subconducting state. For example, it has been shown that the single channel conductance of a calcium channel is affected by the presence or absence of beta and alpha subunits, and the interaction of Ca_{1.4} channels in rods and expression systems with these subunits may differ. Alternatively, the single channel conductance differences may be due to species differences, as transient construct was human Ca_{1.4} while photoreceptor experiments were performed on salamander rods. The salamander genome contains only two L-type channels, while mammals have four L-type channel subtypes.

**Ca_{1.4} voltage-dependent inactivation.** A striking feature of whole-cell current traces with barium as the charge carrier is the slow rate of voltage-dependent inactivation (VDI). For example, after a 10 second depolarization, over 50% and 20% of the peak current remains when the channel is coexpressed with beta_{2a} and beta_{3} respectively, which are significantly greater than Ca_{1.3} and Ca_{1.2} recorded under identical conditions. Indeed, the macroscopic time constants determined for inactivation from whole-cell currents have been measured at 9.9 s (beta_{1b}, 9.8 s (beta_{2a}), 15.4 s (beta_{3}), and 12.3 s (beta_{3}) for Ca_{1.4} channels. However, this time constant does not reflect pure inactivation kinetics, as Ca_{1.4} currents are complicated by the presence of a window current and late channel openings (discussed below). While some reports indicate that the extent of inactivation is independent on beta subunit coexpression (Baumann et al. did not detect difference between beta_{2a} and beta_{3}, McRory et al. did not detect differences among any of the four beta subunits), other reports have detected a difference (Koschak et al. found slightly more inactivation with beta_{3} than beta_{2a}). Regardless, when a 10 second conditioning pulse is given, half-inactivation values measured are -15 mV. Taken together, the inactivation and activation data reveal a substantial window current, which permits ion influx under constant depolarized conditions over a window of approximately 40 mV. While these 10 second conditioning times are similar in magnitude to the measured tau_{inact}^* increasing the depolarization times does not significantly shift the measured V_{1/2} inactivation (V_{1/2} inact 10 seconds = -20.1 ± 1.6 mV, V_{1/2} inact 20 seconds = -21.4 ± 2.1 mV, V_{1/2} inact 50 seconds = -25.7 ± 1.9 mV; no significant difference using ANOVA; data from Baumann et al.).

A slow macroscopic time course of inactivation can arise from several mechanisms, including long opening events, short closing times between events, and late opening events. Cell-attached patch single-channel analysis of Ca_{1.4} currents (coexpressed with beta_{2a} subunits) demonstrate that the slow voltage-dependent macroscopic currents are likely the result of late opening events during sustained depolarization. Recent whole-cell patch clamp studies have demonstrated that the distal C-terminus helps to limit VDI in Ca_{1.4}, as Ca_{1.4} constructs lacking the last 122 amino acids displayed faster and more pronounced inactivation than full length constructs. The exact residues responsible for this remain unidentified and no data is available on the single channel properties of this deletion construct.

**Ca_{1.4} calcium-dependent inactivation.** One hallmark of Ca_{1.1}, Ca_{1.2}, and Ca_{1.3} currents is the presence of calcium-dependent inactivation (CDI), a process that manifests itself as an increase in the rate of inactivation in calcium versus barium (reviewed in refs. 40, 41). CDI is a calmodulin-mediated process that is conserved among high voltage-activated calcium channels, with Ca_{1} members utilizing the high affinity calcium lobe of calmodulin, whereas, the
Ca,2 channels utilize the low affinity binding lobe of calmodulin.39
Surprisingly, expression studies with Ca,1.4 reveal a lack of CDI despite intact EF hand, pre IQ, and IQ domains, irrespective of intracellular calcium buffering conditions.

Ca,1.4 channels are believed to be responsible for tonic glutamate release in rod photoreceptors. This necessitates that the channels support calcium influx under constantly depolarized conditions, and therefore CDI must be limited. Recent studies using expression systems and insights from a mutation implicated in CSNB2 have led to the identification of an autoinhibitory domain in the C-terminus of Ca,1.4 channels that abolishes CDI.38 This domain is comprised of 23 amino acids in the distal C-terminus, in human 40 and mouse 31 Ca,1.4 channels. Using biochemical and FRET techniques, Singh and colleagues demonstrate that this inhibitory domain specifically interacts with the EF-hand, preIQ, and IQ domains to uncouple the CDI-calcmodulin machinery from the channel, thereby preventing CDI in Ca,1.4. Furthermore, this domain can act as an autonomous unit, but does require additional amino acids following the IQ domain 41 (the “A” domain). If the distal C-terminus of Ca,1.4 containing these 23 critical amino acids plus the additional (A domain) residues are transferred to other channels such as Ca,1.2 41 or Ca,1.3,38 these new chimeric channels lack CDI. Thus, Ca,1.4 channels appear to have evolved a unique mechanism that limits both the amount of VDI and CDI they undergo.

Ca,1.4 voltage-dependent facilitation. Ca,1.4 calcium channels display voltage-dependent facilitation that is not G-protein dependent.31 The authors show that peak current increases by 13% (with β3) or 18% (with β2a) when a conditioning prepulse to +80 mV is applied immediately before the test pulse. Although the measured ratio is small, it is significant above background since no facilitation was observed for Ca,2.1 channels recorded under identical conditions. However, this ratio only includes data from those cells that demonstrated facilitation (59% of cells with β3, and 85% of cells with β2a). Those cells which display voltage-dependent facilitation also display increased activation and inactivation kinetics, in agreement with previous studies on other facilitated L-type currents.32

Ca,1.4 pharmacology. Based on sequence similarities, Ca,1.4 channels were originally classified in the L-type family and therefore predicted to be sensitive to dihydropyridine (DHP) antagonists and agonists. These predictions support observations in retinal cells that DHP antagonists reduce calcium currents,14,45 a portion of which are likely the result of the presence of Ca,1.4 channels. Subsequent expression studies have demonstrated that Ca,1.4 channels are indeed blocked in a voltage-dependent manner by DHPs such as isradipine 31 and nifedipine.11 When tested under identical conditions, at more negative potentials Ca,1.4 channels are slightly less sensitive to DHP block than Ca,1.3 channels, and both are significantly less sensitive than Ca,1.2 channels: at more depolarized potentials however, Ca,1.4 and Ca,1.3 channels show similar sensitivities.31 These data support the notion DHP antagonists interact with L-type channels in a voltage-dependent manner (reviewed in ref. 46). The L-type calcium channel DHP agonist BAYK 8644 causes significant enhancement of Ca,1.4 currents, as well as a small hyperpolarizing shift in the current-voltage relation.31,34 At the single channel level, this agonist slightly increases the single channel conductance, and increases the open time constant by -45%,35 but does not cause the appearance of the second mode of gating with longer open time constants characteristic of other Ca,1 channels, suggesting that Ca,1.4 may lack this second mode of gating altogether.

Transiently expressed Ca,1.4 channels are also weakly sensitive to verapamil.34 Baumann et al.35 also show that L-cis-diltiazem and D-cis-diltiazem block Ca,1.4 currents with similar affinities, a feature which appears to be unique to Ca,1.4 among Ca,1 family members, as previous studies have shown higher affinities of D- versus L-cis-diltiazem for other Ca,1 channels (reviewed in ref. 49). Ca,1.4 currents are insensitive to 6-2-conotoxin GVIA at concentrations sufficient to block Ca,2.2 channels, and are highly sensitive to block by divalent metal ions such as cadmium.11 Taken together, these pharmacological data agree with the original classification of Ca,1.4 channels into the L-type family.

Characterization of CSNB2 mutations. Dozens of mutations in CACNA1F have been reported and implicated in CSNB2 and X-linked cone rod dystrophy (CORDX3) phenotypes,9,17,33,40,47,51 and may be either gain or loss of function in nature. Patients with CSNB2 are typically diagnosed based on abnormal electroretinogram (ERG) recordings. The a-wave is reduced and the b-wave is substantially reduced or lacking. Maybe in patients with mutations in CACNA1F, alteration in a- and b-waves are interpreted to be the result of loss of transmission from the rods to the second order neurons, and hence attributable to a loss of Ca,1.4 channel function. However, some CSNB2 patients have normal CACNA1F sequences and mutations in other genes. For example, mutations in the gene encoding for calcium binding protein 4 (CBP4) results in the CSNB2 phenotype.52 Therefore, CSNB2 phenotype may not be due to a mutation in CACNA1F, and characterization of specific Ca,1.4 mutations is necessary to see if there is an effect on channel targeting, trafficking, biophysical properties, or interactions with other retinal proteins to explain the phenotype.

Several of the mutations implicated in CSNB2/CORDX3 have been incorporated into wild-type Ca,1.4 constructs and examined functionally in expression systems, the results of which are summarized in Table 2. While many of these mutations reveal an alteration in the biophysical properties of the channel, some of these mutations do not significantly alter the biophysics of the mutant channel. This in itself does not mean that the mutation cannot be responsible for CSNB2 or CORDX3 phenotypes. For example, the mutation may produce subtle biophysical or targeting effects that are masked in expression systems but produce dramatic differences that alter photoreceptor function. Alternately, the mutation may affect interactions with other proteins typically found in photoreceptors but not included in the expression system. Given the difficulty in obtaining direct electrophysiological recordings from mammalian photoreceptors (thus necessitating the use of expression systems which may lack endogenous signals and proteins of rods or cones), and short of generating (i.e. knock-in) each mutation into mice, we can only hypothesize how the given mutation may function in an intact photoreceptor and lead to clinical phenotypes.

Table 2 summarizes Ca,1.4 mutations tested in mammalian and oocyte expression systems. Two of the mutants, G369D and W1459X, have been tested separately by two groups, but differing results were obtained (see Table 2). A possible explanation for this difference could be the difference between the two Ca,1.4 clones tested. For example, the clone used by Hoda et al.35 differs from that used by McRory et al.11 with respect to two splice variants in the I-I linker and IVS3-IVS4 linker, and with four amino acid substitutions (K58R, E380G, R1282H, I1306V; in both cases, each group confirmed their original sequence from several clones, indicating that the difference is not likely due to PCR or cloning errors).11,31 As discussed by Hoda et al.,35 the E380G change is in close proximity
### Table 2  **Summary of CACNA1F mutations implicated in clinical phenotypes, and the effects observed when the mutations were tested in expression systems**

| Mutation   | Phenotype                                                                 | Reference                                      |
|------------|---------------------------------------------------------------------------|------------------------------------------------|
| S229P      | no currents recorded, but protein staining positive                        | Hoda et al. 33                                 |
| G369D      | increased voltage-dependence of inactivation in Ba\(^{2+}\), \(V_{1/2} \text{ Ca}^{2+}\) is shifted to right | McRory et al. 11                               |
| (G369D)    | (\(V_{1/2} \text{ Ca}^{2+}\) and Ba\(^{2+}\) shifted to left, increase time to peak current, slowed inactivation, shift in reversal potential) | (Hoda et al. 33)                              |
| R508Q      | NSD in biophysics; decrease in channel density at membrane (temperature dependent) | Hoda et al. 33, 40                            |
| G674D      | NSD in biophysics                                                          | McRory et al. 11                               |
| (G663D)    | (NSD in biophysics)                                                        | (Hoda et al. 33)                               |
| I745T      | \(V_{1/2} \text{ (Ba}^{2+}, \text{ Ca}^{2+}\) shifted to left and increased current density, slower voltage-dependent inactivation | Hemara-Wahanui et al. 17                       |
| A928D      | NSD                                                                        | McRory et al. 11                               |
| (A917D)    | (NSD in biophysics)                                                        | (Hoda et al. 33)                               |
| L1068P     | currents only with BayK8644 (\(V_{1/2} \text{ Ca}^{2+}\) shift to left, rate of inactivation slower in Ba\(^{2+}\) but not Ca\(^{2+}\)) | Hoda et al. 33                                 |
| L1364H     | NSD in biophysics except change in reversal potential; faster inactivation in Ba\(^{2+}\) but not Ca\(^{2+}\), increase in recovery from inactivation in Ca\(^{2+}\) and Ba\(^{2+}\), decrease in channel density at membrane (temperature dependent) | Hoda et al. 33                                 |
| W1459X     | NSD                                                                        | McRory et al. 11                               |
| (W1440X)   | (no currents recorded, protein staining negative)                          | (Hoda et al. 33)                               |
| K1591X     | CDI sensitive, activation shifted to left, inactivation shifted to left    | Singh et al. 38                                |

NSD denotes "not significantly different" when biophysics compared to wild-type Ca\(_{1.4}\). \(V_{1/2}\) denotes half activation potential. \(V_{1/2, \text{ inactivation}}\) denotes half inactivation potential. When characterization of identical mutations was carried out by different groups, the results are presented together and bracketed for clarity. Note however, that mutation numbers are as reported in each paper, and may differ between groups because of splice variants and differences in Ca\(_{1.4}\) clone numbering.

Data is lacking as to whether human CSNB2 patients also display these retinal morphological defects.

### Calcium Currents Recorded in Retinal Preparations: Possible Involvement of Ca\(_{1.4}\)

Characterization of non-mammalian rod and cone calcium currents. Calcium currents recorded from photoreceptors in the past were typically recorded in non-mammalian vertebrate photoreceptors because the large sizes of the rods and cones permit relatively easy access. Early studies using single micropipette voltage-clamp to record from salamander rods reveal sustained calcium currents that fully activate in less than 20 ms and do not begin to decay for several seconds.\(^5^7\) These currents activated at voltages more positive than ~45 mV and peaked around 0 mV (half activation potential, \(V_{1/2, \text{ activation}}\) ~ 22 mV). Subsequent studies using whole-cell patch clamp produced similar results (\(V_{1/2, \text{ activation}}\) ~31 mV), as well as demonstrating that the currents were sensitive to Cd\(^{2+}\) and were larger when barium replaced calcium as charge carrier by 10–20%.\(^5^8\) In these experiments, CdI was observed only when internal calcium buffering with EGTA was lowered from 10 mM to 0.1 mM. Similar results were obtained from experiments with cones from lizards in which currents activate positive to ~40 mV, peak around 0 mV, and do not show CdI unless internal calcium buffering is minimized.\(^5^9\) Furthermore, these currents were also shown to be sensitive to DHP antagonists such as nitrrendipine and agonists such as BayK 8644. Taken together, these results suggest that the voltage-sensitive calcium currents in rods and cones are likely mediated by L-type calcium channels, 60,61 and have since been verified by numerous other studies.\(^5^9,62-65\)

A quantitative comparison of calcium currents in salamander rods and cones using 3 mM Ca\(^{2+}\) as charge carrier showed that current in both cell types begins to activate near ~40mV and peaks near 0 mV.\(^6^5\) No significant differences in biophysical properties...
were noted between different types of photoreceptors (rod $V_{i0} \sim -13.3 \pm 3.1$ mV, cone $V_{i0} \sim -15.3 \pm 2.3$ mV), and both were sensitive to DHPs. A subsequent study by Wilkinson and Barnes provides evidence that the currents in the two types of photoreceptors may be mediated by multiple calcium channel subtypes. For example, 5 µM nisoldipine and 10 µM nifedipine failed to eliminate all cone calcium current, and 15% current was shown to be reversibly blocked by ω-conotoxin GVIA. The current in rods was less sensitive to that in cones, and neither rod nor cone calcium currents were sensitive to P-type calcium channel blocker ω-agatoxin IVA.

The biophysical properties match quite well with those for transiently expressed Ca$_{1.4}$ currents previously described, although precise determination cannot be made since these cells also likely express Ca$_{1.3}$ channels. Both Ca$_{1.4}$ and Ca$_{1.3}$ share some similar biophysical properties in expression systems, making definitive assignment of photoreceptor currents difficult based on expression system data. The slow inactivation kinetics support Ca$_{1.4}$ currents over Ca$_{1.3}$ currents, although a recent study suggests that Ca$_{1.3}$ channels may not inactivate in the presence of CBP4. Furthermore, while many reports have suggested that the calcium currents recorded from rods and cones display slow inactivation kinetics and CDI only when internal calcium buffering is minimized, other studies have shown that photoreceptor currents can inactivate. Calcium currents recorded from isolated rod photoreceptors bathed in physiological calcium concentrations (1.8 mM) inactivate almost completely when held at a test depolarization of -10 mV for 5 seconds, while cells stepped to -40 mV for 5 seconds (representing the physiological dark resting membrane potential of rods) displayed less than 15% inactivation. Inactivation time courses from rods were fitted with a single exponential, with $\tau_i$ ranging from 1.29 s to 2.18 s, values which are approximately 3-fold smaller than for transiently expressed Ca$_{1.4}$.  

Depolarization-induced facilitation of the DHP-sensitive current in photoreceptors has also been investigated. Calcium channel currents were shown to increase or facilitate with application of strong pre-pulse depolarization. Rod photoreceptors displayed facilitation values of -21% with ruptured-patch recordings. These values are on par with those recorded from Ca$_{1.4}$ expression experiments but cannot be distinguished from Ca$_{1.3}$, Ca$_{1.4}$, and Ca$_{1.4}$ facilitation ratios. This facilitation is entirely voltage-dependent and does not involve G proteins (just as for transiently expressed Ca$_{1.4}$).  

Characterization of mammalian rod and cone calcium currents. Due to their small size, mammalian rod and cone photoreceptors are less well characterized than their non-mammalian vertebrate counterparts. Characterization of porcine photoreceptor currents required subtracting the currents obtained in the presence of total calcium block with cobalt from those in the presence of barium, and revealed very small currents that activate at test potentials positive to -70 mV, and peak around -25 mV. The currents were also sensitive to calcium channel antagonists such as D-cis-diltiazem, L-cis-diltiazem and verapamil (recall similar pharmacological profile for transiently expressed Ca$_{1.4}$ channels). Similarly, murine photoreceptor recordings give $V_{i0} \sim -35$ mV.  

Recordings from macaque cones demonstrate that currents begin to activate -60 mV and peak at -30 mV, while those of the tree shrew were slow inactivating, display $V_{i0} \sim -40$ mV, and are sensitive to nifedipine but not calcisepine, nimodipine or nicardipine. The authors also reported that at the dark resting potential of -40 mV, only 50% of the channels were activated. In summary, currents recorded from photoreceptors share many of the characteristics of transiently expressed Ca$_{1.4}$ channels (and Ca$_{1.3}$ channels), and the presence of at least both of these channel subtypes in photoreceptors makes definitive assignment difficult.

Transiently expressed Ca$_{1.4}$ currents often appear depolarized relative to the typical resting membrane potential of -40 mV in dark adapted mammalian rods. The position of the I–V relation is sensitive to external and internal recording solutions; typically in transient expression systems data are recorded with barium as the charge carrier. Upon switching to physiological levels of calcium, the I–V relation shifts in the depolarizing direction. Furthermore, the expression systems do not have all the proteins found in photoreceptors, and at least one of these proteins (CBP4) shifts the I–V relation substantially in the hyperpolarizing direction. Taken together, when transiently expressed Ca$_{1.4}$ channels are recorded in physiological calcium levels and calcium binding protein 4 is also coexpressed, the resulting I–V relation mirrors that obtained from recording in photoreceptors.

Modulation of calcium currents recorded from photoreceptors. Several compounds have been shown to affect calcium currents recorded from photoreceptors. For example, application of a nitric oxide (NO) generating substance S-nitrosocysteine (SNC) in salamander rod photoreceptors increases calcium current over a broad range of step potentials, and shifts the current-voltage relation to hyperpolarized values by approximately 5 mV. When the effects of NO were studied in salamander cone photoreceptors, inhibitory and depolarizing effects were observed. To date, the effect of nitrogen-donor species on Ca$_{1.4}$ currents in expression systems has not been studied.

Application of caffeine to salamander rod photoreceptors reversibly suppresses peak calcium current by up to 50% with no apparent shift in the I–V relation. The release of endogenous glutamate from rods was also shown to be depressed by caffeine addition, suggesting a possible effect on Ca$_{1.4}$ channels. Adenosine also appears to have inhibitory effects on rod calcium current, inhibiting the peak current-voltage relation by -23% again without affecting the overall activation voltage. This inhibitory effect by adenosine appears to be mediated through the A2 adenosine receptor when selective agonists for A2 are used, and not through A1 nor A3 receptors. The effects of caffeine and adenosine on transiently expressed Ca$_{1.4}$ channels remains to be examined, as do the effects of dopamine, cAMP, and PKA activity. Dopamine acting through D2 receptors modulates calcium currents in photoreceptors, enhancing currents in rods and inhibiting currents in cones. In the same report, effects of cAMP and PKA activity on the photoreceptors were examined. Sp-cAMPS and forskolin both activate PKA and inhibit calcium currents from rod and small single cones, while enhancing calcium currents in large single cones.

Hormones are also known to modulate photoreceptor calcium currents. For example, insulin reversibly inhibits calcium currents in salamander rod photoreceptors by -20%. Pre-treatment of an insulin receptor with the specific tyrosine kinase inhibitor HNMPA-AM$_2$ prevents receptor tyrosine kinase autophosphorylation, suggesting the receptor tyrosine kinase is required for insulin inhibition in rod photoreceptors. Similarly, activation of SST receptors by somatostatin in photoreceptors differentially modulate calcium currents. Application of somatostatin reduces rod calcium currents by 33% and increases cone currents by 40%. Both effects are mediated through G-protein activation that can be blocked with the application of pertussis toxin, suggesting a G$_{i/o}$ mediated pathway.  

Long chain polyunsaturated fatty acids have a potent effect in
suppressing calcium current from salamander rod photoreceptors. Endogenous species such as retinoids, including retinal, retinol and retinoic acid are enriched in photoreceptors and could inhibit Ca\textsubscript{1,4} channels if they are colocalized. Finally, cannabinoid CB1 receptors have been detected in the photoreceptor terminals of several animals including salamander, chick and monkey. Activating CB1 receptors with WIN55212-2 enhanced calcium currents of rod photoreceptors by 39% and decreased calcium currents of large single cones by 50%. It is not clear whether this is an important modulator of calcium channels in photoreceptors, including Ca\textsubscript{1,4}. Further studies are required to test the effects of these compounds which modulate calcium currents in photoreceptors on transiently expressed Ca\textsubscript{1,4} (and Ca\textsubscript{1,3}) to examine modulation.

CONCLUSIONS

The CACNA1F gene is expressed in numerous tissues throughout the body, suggesting that this channel may play a larger role in human physiology beyond function in photoreceptors. Unlike other calcium channels, the CACNA1F gene appears to be highly susceptible to mutations, with resulting phenotypes affecting visual function. However it is not clear at this time if patients with these mutations also present immune and some endocrine gland dysfunction, given the expression profiles of Ca\textsubscript{1,4} mRNA and protein. Expression studies have given insight into unique properties of the channel, including gating parameters, pharmacology, and intrinsic structural features which limit activation of the channel. These features permit Ca\textsubscript{1,4} to support sustained calcium influx resulting in tonic glutamate release at photoreceptor ribbon synapses. Furthermore, while several of the mutations have been characterized in expression systems, effects of the mutations have not been described in native intact cells, due to the difficulties in recording from mammalian (human) photoreceptors. Two CACANA1F knockout mouse strains have been described, and these may play a role in future studies to investigate wild-type and mutant Ca\textsubscript{1,4} function in native cells such as photoreceptors and T lymphocytes.

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