Functional Implications of a Novel EA2 Mutation in the P/Q-Type Calcium Channel

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Episodic ataxia type 2 (EA2) is an autosomal dominant condition characterized by paroxysmal attacks of ataxia, vertigo, and nausea, typically lasting minutes to days in duration. These symptoms can be prevented or significantly attenuated by the oral administration of acetazolamide; however, the mechanism by which acetazolamide ameliorates EA2 symptoms is unknown. EA2 typically results from nonsense mutations in the CACNA1A gene that encodes the α_{1A} ($Ca_v2.1$) subunit of the P/Q-type calcium (Ca^{2+}) channel. We have identified a novel H1736L missense mutation in the CACNA1A gene associated with the EA2 phenotype. This mutation is localized near the pore-forming region of the P/Q-type Ca^{2+} channel. Functional analysis of P/Q-type channels containing the mutation show that the H1736L alteration affects several channel properties, including reduced current density, increased rate of inactivation, and a shift in the voltage dependence of activation to more positive values. Although these findings are consistent with an overall loss of P/Q-type channel function, the mutation also caused some biophysical changes consistent with a gain of function. We also tested the direct effect of acetazolamide on both wild-type and H1736L mutated P/Q-type channels and did not observe any direct action on channel properties of this pharmacological agent used to treat EA2 patients.

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Episodic ataxia type 2 (EA2) is an autosomal dominant condition characterized by paroxysmal attacks of ataxia, vertigo, and nausea, lasting minutes to days in duration.[1,2] Most EA2 cases can be either prevented or significantly attenuated by the oral administration of acetazolamide.[3] However, the mechanism by which acetazolamide ameliorates EA2 symptoms is unknown. EA2 can result from mutations in the CACNA1A gene that encodes the α_{1A} ($Ca_v2.1$) subunit of the P/Q-type calcium (Ca^{2+}) channel. P/Q-type Ca^{2+} channels are widely distributed throughout the central nervous system and are the most abundantly expressed subtype of Ca^{2+} channel in the cerebellar Purkinje cells where they mediate most of the depolarization-induced Ca^{2+} currents.[4]

To date, 30 different mutations associated with EA2 have been described in the CACNA1A gene.[2] Most are nonsense mutations resulting in a truncated protein product. For example, the R1820X nonsense mutation results in a premature stop and loss of the α_{1A} wild-type carboxyl-terminal tail region. When expressed in *Xenopus oocytes*, P/Q-type channels containing the R1820X mutation do not form functional Ca^{2+} channels.[5] Interestingly, despite being nonfunctional, the mutated channel was found to have a dominant negative effect on coexpressed wild-type α_{1A} channels. Electrophysiological studies of the missense mutation F1491S also have demonstrated nonfunctional channels, resulting in the hypothesis that CACNA1A gene mutations associated with EA2 lead to a complete loss of channel function.[6] In contrast, another study demonstrated that the EA2 mutation AY1593/1594D was able to form functional channels when expressed in *X. laevis* oocytes, resulting in decreased inward mediated Ca^{2+}.[7] However, there was failure of expression of this same mutant channel in mammalian systems (HEK 293 cells), still leaving the question of whether human EA2 α_{1A} mutations can form functional channels.

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In this study, we have identified a novel EA2 mutation in the CACNA1A gene, H1736L. We have also demonstrated functional expression of the H1736L EA2 mutation in the HEK 293 (HEK) cell mammalian expression system and have found that the mutation significantly alters several P/Q-type channel biophysical properties. As the carbonic anhydrase inhibitor acetazolamide reversed the clinical symptoms in patients with this mutation, we also examined the effects of direct application of acetazolamide on both wild-type and mutated Ca^{2+} channels exogenously expressed in HEK cells.

Case Report

Identification of Novel Mutation

PATIENT. The patient presented at the age of 11 years with episodes of ataxia and nystagmus lasting minutes in duration. These episodes were alleviated with acetazolomide. The patient's interictal examination was normal. At the age of 22 years, the patient still experienced episodes if she stopped her medication. The patient's father was reported to have had similar episodes but was unavailable for consultation.

MUTATION ANALYSIS. DNA was obtained from the patient and her mother after informed consent. Primers were used as previously described[8] to polymerase chain reaction (PCR) amplify the intron/exon boundaries of the 47 exons of the CACNA1A gene. After PCR amplification, heteroduplex analysis (HA)[8] was performed, and single mismatch pairs were subjected to automated sequencing using an ABI 377 automated DNA sequencer (Prism Big Dye Terminators Cyclic Sequencing kit; ABI Applied Biosystems, Foster City, CA). The sequence generated was compared with 100 control samples and the human genomic sequence for the CACNA1A gene in the GenBank database.

SITE-DIRECTED MUTAGENESIS. Utilizing standard PCR-based in vitro mutagenesis, the wild-type human α_{1A} ($Ca_v2.1$) subunit was altered to contain the H1736L mutation.[9] The long form (isoform 2) of the human α_{1A} was used as the wild-type source cDNA; this form encodes in-frame translation of the nonexpanded polyglutamine tract. The full-length construct was cloned into pcDNA3.1 Zeo(+). The *Eco*RV to *Xho*I fragment of the human α_{1A} construct was subcloned into pBluescript II KS+ vector and subjected to mutagenesis. The H1736L substitution was obtained according to protocol using the QuikChange (Stratagene, La Jolla, CA) method with paired forward and reverse mutagenesis primers. The fragment was inserted into the wild-type full-length human α_{1A} cDNA in pcDNA3.1 Zeo(+). The mutant H1736L full-length construct was confirmed by direct DNA sequencing.

CELL CULTURE AND TRANSFECTION. HEK cells were cultured as previously described.[10] The HEK cells were transiently transfected with wild-type or H1736L human α_{1A} (6μg in pCDNA3.1 vector) along with β_{1b}, α_2 and the CD8 marker plasmid at a 1:1:1: 0.25 molar ratio using Lipofectamine (Invitrogen, La Jolla, CA). To ensure valid current density and transfection efficiency comparisons, we always transfected wild-type and mutant human α_{1A} plasmids at the same time and electrophysiological recordings alternated between the channel types.

ELECTROPHYSIOLOGICAL RECORDING. Macroscopic currents were recorded using the whole-cell patch-clamp technique.[11] The external recording solution contained 2mM $BaCl_2$, 1mM $MgCl_2$, 10mM Hepes, 40mM TEACl, 92mM CsCl, and 10mM glucose, pH 7.2. The internal pipette solution contained 120mM $CsCl_2$, 11mM EGTA, 10mM Hepes, 2mM Mg-ATP, pH 7.2. Recording conditions, apparatus, pipettes, and analysis software were as previously described.[10] Only currents greater than 100pA were analyzed.

RECORDING PROTOCOLS AND DATA ANALYSIS. The voltage dependence of activation was measured by a series of 80 milliseconds depolarizing pulses applied from a holding potential of −100mV to membrane potentials from −50 to +25mV, increasing at 5mV increments. The potential that elicited peak currents ("peak potential", ranging from −15 to 0mV) was obtained from this protocol and used in subsequent protocols. The holding potential of −100mV also was used in all other protocols.

Current–voltage relationships were fitted with the modified Boltzmann equation: $I = [G_{max}*(V_m - E_{rev})]/[1+\exp(\{V_m - V_{50act}\}/k_a)]$, as previously described.[10] Activation and inactivation rates during steps to peak potential were well described by single exponential curves to give τ_{act} and τ_{inact} values, respectively. Steady state inactivation curves were fit with a Boltzmann equation: $I/I_{max} = [1 + \exp(\{V - V_{50inact}\}/k_i)]^{-1}$ as previously described.[10]

To examine cumulative inactivation during a train of pulses, we measured peak current during a 1Hz train of 15 pulses. Each pulse was to peak potential and 100 milliseconds in duration. The percentage of current decay (% decay) was determined by comparing the 15th pulse to the first pulse.

Recovery of currents from short inactivation was tested using a double-pulse protocol. The first depolarizing pulse was to peak potential for 2 seconds, followed by a repolarization (−100mV) period of variable length. This "interpulse interval" ranged from 10

to 10,240 milliseconds using a doubling scale. Immediately after this interval, a second 200-millisecond test pulse to peak potential occurred, followed by a 30-second recovery period. The peak current from the second test pulse was normalized to the first 2-second pulse, and the plot of normalized current versus repolarization time was fit with a biexponential equation to give time constants for the fast (τ_{fast}) and slow (τ_{slow}) components of recovery. Statistical significance was determined by Student's *t* tests.

ACETAZOLAMIDE APPLICATION. Acetazolamide was obtained from Sigma-Aldrich (Ontario, Canada), and 100mM concentrated stock solutions were prepared in dimethylsulfoxide on each day of experiments. The syringes containing the acetazolamide recording solutions were wrapped in foil throughout the experiments to prevent degradation of the acetazolamide due to light exposure. The highest concentration of dimethylsulfoxide in the recording solution did not exceed 0.1%, a concentration that did not detectably affect Ca^{2+} channel properties. For acetazolamide cell culture experiments, transfected HEK cells were incubated at 37°C with 10μM acetazolamide for 1 to 4 hours immediately before electrophysiological recordings. The media used for this incubation (Invitrogen) contained no pH buffer and contained, 3.7 g/L $NaHCO_3$, providing substrate for the targeted carbonic anhydrase enzyme.

Results

Mutational Analysis

Heteroduplex analysis of the CACNA1A gene from genomic DNA of the EA2 patient showed a migration mismatch in exon 34 (Fig 1A). Subsequent direct DNA sequence analysis demonstrated an A to T substitution in exon 34. The mutation resulted in the substitution of a positively charged histidine 1736 for a neutrally charged leucine in the putative extracellular linker in domain IV between the S5 and S6 transmembrane regions (see Fig 1B). This mutation was not found in the mother. The father who was reportedly symptomatic was not available for genetic analysis.

Electrophysiology Studies

THE H1736L MUTATION CAUSES A REDUCTION IN THE EXPRESSION OF FUNCTIONAL P/Q-TYPE CA^{2+} CHANNELS. The H1736L mutation was seen to impair, but not completely block, expression of functional P/Q-type Ca^{2+} channel currents in transfected HEK cells. The H1736L mutation significantly ($p < 0.001$) reduced the percentage of cells patched that had detectable currents from 48.5 ± 3.9 % (11 experiments, 116 cells tested) for wild type to 19.9 ± 2.8 % (11 experiments, 151 cells tested) for H1736L channels (Table 1). In addition to impairing transfection efficiency, the

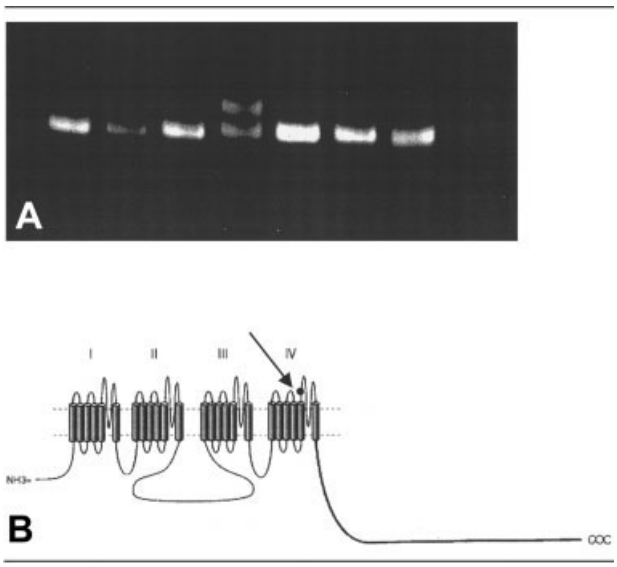

Fig 1. (A) Heteroduplex analysis. Image of a heteroduplex gel. The double band in the center contains the heteroduplex mismatch, which subsequently was demonstrated to contain an A to T mutation on direct sequencing. The single bands on either side are the controls. (B) Schematic representation of the α_{1A} subunit of the P/Q-type Ca^{2+} channel. The arrow indicates the extracellular region of the channel where the H1736L mutation is located.

H1736L mutation also reduced current density, which is proportional to the number of functional channels per surface area of the transfected cells. The peak current density of wild-type channels (−103.0 ± 16.6pA/pF; n = 26) was significantly larger ($p < 0.001$) than the current density of H1736L channels (−38.2 ± 6.1pA/pF; n = 31; Fig 2A; see Table 1).

THE H1736L MUTATION CAUSES LOSS-OF-FUNCTION VOLTAGE-DEPENDENT AND KINETIC CA^{2+} CHANNEL EFFECTS. Current–voltage relations were measured to determine the membrane potential at which half of the channels open (V_{50act}). The H1736L mutation was found to significantly ($p < 0.01$) shift the voltage dependence of channel activation by approximately 4mV in the depolarizing direction (see Fig 2B; Table 1). In contrast, the H1736L did not significantly affect the voltage dependence of inactivation ($V_{50inact}$) (see Table 1). The H1736L mutation also altered Ca^{2+} channel kinetics. The rate of Ca^{2+} channel inactivation (τ_{inact}) was significantly ($p < 0.001$) increased for H1736L currents (Fig 3, Table 1). However, the rate of Ca^{2+} channel activation was not significantly affected by the H1736L mutation (see Fig 3, Table 1).

THE H1736L MUTATION DECREASES INACTIVATION-DEPENDENT DECAY AND INCREASES RECOVERY FROM INACTIVATION. To measure the amount of Ca^{2+} channel current decay caused by repetitive firing, we used a

Table 1. Biophysical Properties of Wild-Type and H1736L α_{1A} Channels

	Wild-type α_{1A}			H1736L α_{1A}		
Parameter (U)	Mean	SE (±)	n	Mean	SE (±)	n
Expression properties						
Transfection efficiency (%)	48.5	3.9	11	19.9[a]	2.8	11
Current density (pA/pF)	−103.0	16.6	26	−38.2[a]	6.1	31
Voltage-dependant properties						
V_{50act} (mV)	−12.5	1.0	27	−8.3[a]	1.0	23
$V_{50inact}$ (mV)	−60.4	1.5	17	−58.6	1.9	10
Kinetic properties						
τ_{act} (msec)	0.72	0.03	29	0.70	0.04	30
τ_{inact} (msec)	70.2	3.1	30	49.6[a]	2.3	29
Inactivation properties						
decay (%)	30.6	3.6	11	13.8[a]	4.5	10
Recovery − τ_{fast} (msec)	379	62	9	197[b]	15	8
Recovery − τ_{slow} (msec)	3032	358	9	2851	284	8

[a]Significant at $p < 0.0$
[b]Significant at $p < 0.05$

SE = standard error.

1Hz train of 15 100-millisecond pulses. The H1736L mutation was found to have a significant ($p < 0.01$) reduction in the level of current decay compared to wild-type channel current decay (Fig 4, Table). A double-pulse protocol also was used to determine the time constants for current amplitude recovery from a short (2-second) inactivating prepulse. The H1736L channels displayed a significant ($p < 0.05$) increase in the rate of the fast component (τ_{fast}) of this recovery compared with wild-type channels (Fig 5, Table 1), whereas the rate of the slow component of recovery (τ_{slow}) was not significantly altered (see Table 1).

ACETAZOLAMIDE DOES NOT ALTER P/Q-TYPE CHANNEL PROPERTIES THROUGH ph-INDEPENDENT OR ph-DEPENDENT MECHANISMS. The direct (pH-independent) effects of acetazolamide on wild-type P/Q-type channels was investigated by directly perfusing on various concentrations of the drug after appropriate control recordings (2mM Ba extracellular solution perfusion). The presence of 10mM Hepes in both the intracellular and extracellular solutions ensured that any pH-dependent, acetazolamide-mediated indirect effects were eliminated. Under these conditions, perfusing 10μM acetazolamide was found to have no significant effect on the size of the current amplitude, or the voltage-dependent and kinetic properties of the wild-type channels (Table 2).

To test the pH-dependent, indirect effects of acetazolamide on wild-type P/Q-type Ca^{2+} channel activity, we incubated transfected cells in 10μM acetazolamide at 37°C for 1 to 4 hours immediately before recordings. The incubations were performed in a media that was not pH-buffered and that contained $NaHCO_3$. Once again, incubation in 10μM acetazolamide had no significant effects on the voltage-dependent and kinetic properties of the wild-type channels (see Table 2). Therefore, 10μM acetazolamide does not appear to directly alter the biophysical properties of the wild-type P/Q-type Ca^{2+} channels through either pH-dependent, or pH-independent mechanisms.

Discussion

We have identified a novel missense mutation in the CACNA1A gene associated with a classic EA2 phenotype.[1,2] The mutation (A to T in exon 34) results in the substitution of a positively charged histidine 1736 for a neutrally charged leucine in the putative extracellular linker in domain IV between the S5 and S6 transmembrane regions. The mutation was not identified in 100 control samples nor was it reported as a polymorphism in the GenBank database. The mutation lies in close proximity to the putative pore region of the α_{1A} subunit, and it was predicted that this amino acid substitution might have an impact on channel function. The patient who harbored this mutation improved with treatment with a carbonic anhydrase inhibitor, acetazolamide. The proband's mother did not have the mutation. The mutation was presumably inherited from the symptomatic father, but this cannot be stated definitely because he was unavailable for genetic testing.

In one instance, a missense mutation associated with an EA2 phenotype was previously expressed but found to be nonfunctional.[6] In contrast, another study was able to demonstrate functional EA2 mutated channels in *X. laevis* oocytes but not in HEK cells,[7] calling into question whether human EA2 mutations are actually functional when expressed in mammalian systems. This

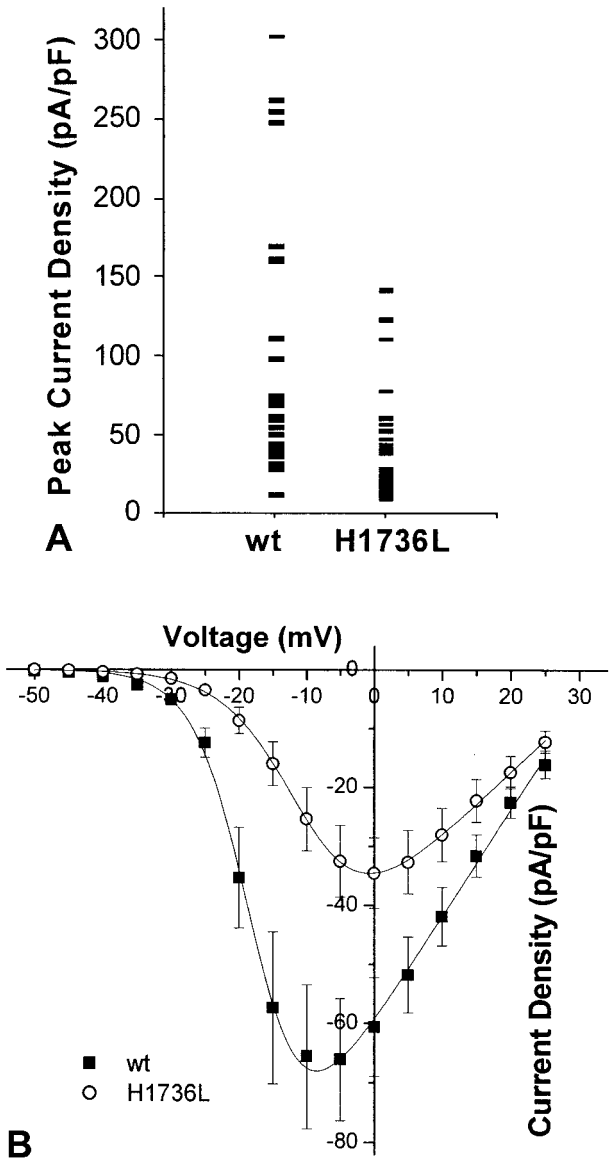

Fig 2. (A) Distribution of cellular current densities (absolute values) for wild-type (wt) and H1736L. (B) Current–voltage relationship. The current density was calculated by determining the peak current at each voltage step divided by the cellular capacitance. The H1736L channel is represented by open circles and the wild-type channels by filled squares. The H1736L channels demonstrate a decrease in current density and a significant ($p < 0.01$) positive shift in the voltage dependence of activation compared with wild-type channels (wt $V_{50act} = -12.5 \pm 1.0$mV, $n = 27$; H1736L $V_{50act} = -8.3 \pm 1.0$mV, $n = 23$). Data points are given as means ± SE.

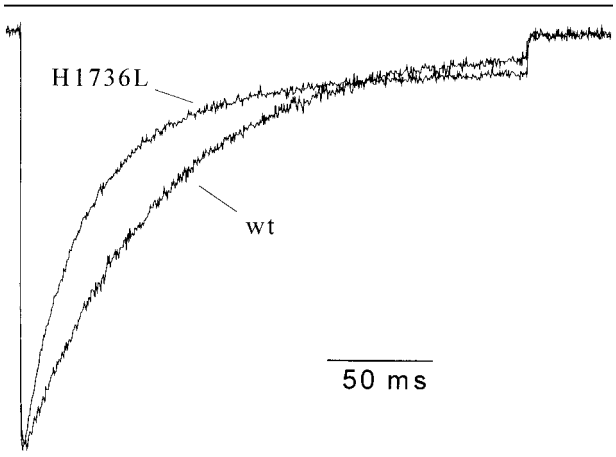

Fig 3. Representative current traces for wild-type (wt) and H1736L channels during a 240-millisecond depolarizing step to peak potential from a holding potential of −100 mV. The H1736L mutation had no significant effect on the rate of channel activation (wt $\tau_{act} = 0.72 \pm 0.03$ milliseconds, $n = 29$; H1736L $\tau_{act} = 0.70 \pm 0.04$ milliseconds, $n = 30$). The H1736L mutation caused a significant ($p < 0.001$) increase in the rate of channel inactivation ($\tau_{inact} = 49.6 \pm 2.3$ milliseconds, $n = 29$) compared with wild-type channel inactivation ($\tau_{inact} = 70.2 \pm 3.1$ milliseconds, $n = 30$).

is the first study to our knowledge to demonstrate functional human EA2 mutated channels in a mammalian system.

The H1736L mutation has three effects on P/Q-type Ca^{2+} channel properties: (1) decreased current in HEK cells (as determined both by the percentage of cells expressing functional channels, and by a decrease in current density). (2) A loss of function effect through voltage-dependent and kinetic Ca^{2+} channel property changes. (3) The mutation decreased inactivation-dependent current decay and increased rate of recovery from inactivation.

Taken together, the effects (1) and (2) would result in a significant reduction in the contribution of the mutated channel proteins to α_{1A} ($Ca_v2.1$)–mediated inward current. Previous studies have failed to demonstrate functional EA2-mutated channels in mammalian systems and have concluded that EA2 mutations result in a complete loss of function. Our results remain consistent with a loss of function hypothesis resulting from a reduction in inward mediated Ca^{2+} current. However, the effect (3) above could result in an increase in inward mediated current. The apparent oppositional effects caused by CACNA1A mutations has been observed previously,[12] and the impact of the H1736L mutation in vivo is likely the net effect of these changes. Because of the drastic decrease in current density (>2.5-fold) for the H1736L mutant channel, we believe the net effect of this EA2 mutation is a loss of function.

Addressing the issue of clinical treatments specifically aimed at the mechanistic basis for channelopathies is highly relevant to a variety of human neurological disorders. It has long been recognized that acetazolamide, a carbonic anhydrase inhibitor, can attenuate EA2 symptoms,[3] although its mechanism of action in alle-

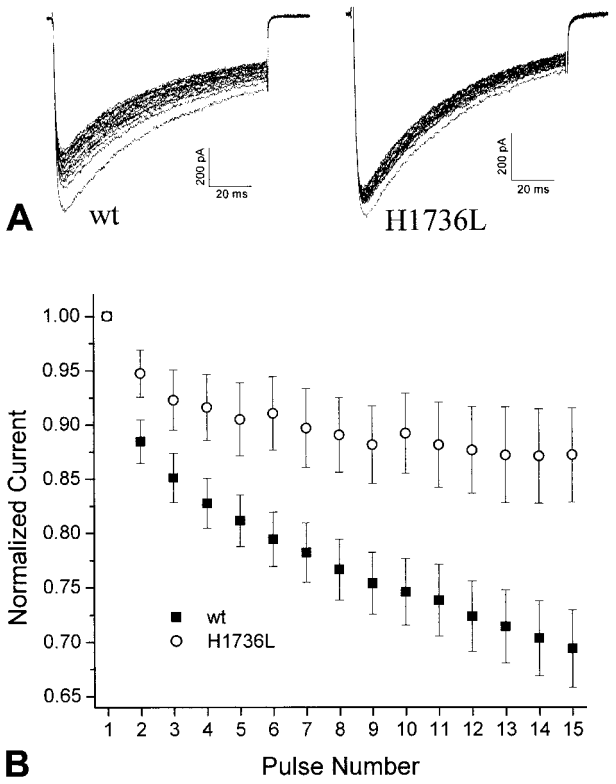

Fig 4. (A) Representative series of current traces for wild-type (left) and H1736L (right) channels during a 1Hz train of 15 100-millisecond test pulses to peak potential. (B) Normalized current value calculated by determining the peak current during a given pulse in the 1Hz train (shown in A) divided by the peak current of the first pulse, for each cell tested. A plot of normalized current versus pulse number was averaged from all cells for wild-type (filled squares) *and H1736L* (open circles) *channels. The H1736L channel showed a significant* ($p < 0.01$) *decrease in current decay after the 15th pulse (13.8 ± 4.5%, n = 10) compared with wild-type channel current decay (30.6 ± 3.6%, n = 11).*

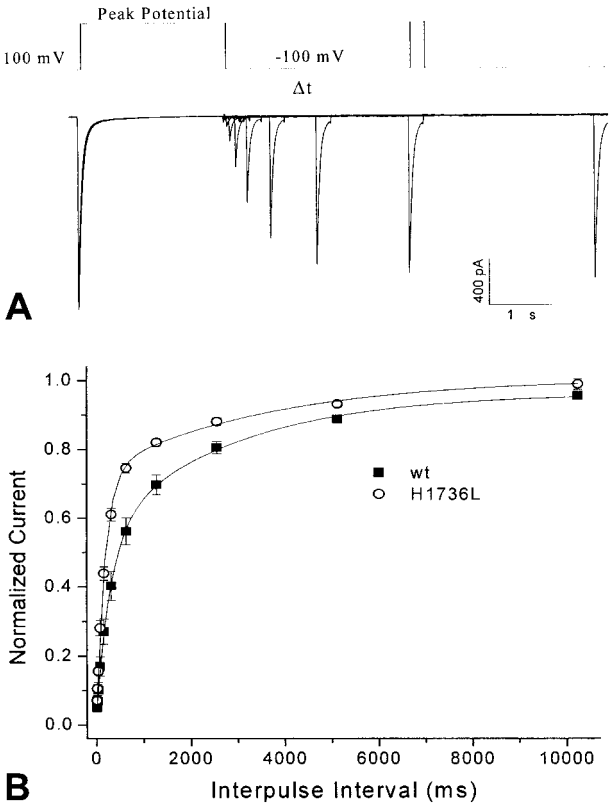

Fig 5. (A) Example waveform representing the rate of recovery from inactivation measured using a double-pulse protocol involving a 2-second depolarizing prepulse to peak potential, a repolarized interpulse interval of variable length, and a 200-millisecond depolarizing test pulse to peak potential. The representative traces for the wild-type channel demonstrates the increase in peak current (recovery) as the interpulse interval is lengthened (bottom). (B) Peak currents during test pulses normalized to the peak currents of their respective prepulses. All values for a specific interval length were averaged for the wild-type (filled squares) and H1736L (open circles) channels. When these recovery curves were fit with biexponential equations, the H1736L channel was seen to have a significantly ($p < 0.05$) *faster fast component of recovery from inactivation compared to the wild-type channel (wt* $\tau_{fast} = 379 \pm 62$ *milliseconds, n = 9; H1736L* $\tau_{fast} = 197 \pm 15$ *milliseconds, n = 8), whereas the slow component of recovery was not significantly different between the channel types (wt* $\tau_{slow} = 3032 \pm 358$ *milliseconds, n = 9; H1736L* $\tau_{slow} = 2851 \pm 284$, *n = 8). Data points are given as means ± SE.*

viating EA2 symptoms associated with defects in the CACNA1A gene is currently unknown. Because most of the EA2 mutations result in a truncated protein product and a previous study has demonstrated truncated channels to be nonfunctional,[5] one possibility is that acetazolamide elicits its effect on wild-type P/Q-type channels. One hypothesis is that acetazolamide influences wild-type channels by causing a shift in the voltage dependence of activation to more negative voltages that, in turn, facilitates channel opening and compensates for the reduced inward mediated Ca^{2+} resulting from the mutation. Functional examination of the effects of acetazolamide on both wild-type and mutant P/Q-type Ca^{2+} channels does not support this hypothesis. Our results suggest that acetazolamide does not directly alter P/Q-type Ca^{2+} channel properties through either pH-independent or pH-dependent mechanisms.

We tested the direct effects of acetazolamide on whole-cell patch recordings of wild-type channels with Hepes buffer in both the extracellular bath solution and intrapipette solution and did not observe any changes in the biophysical properties of the wild-type channels. In particular, there were no changes in the voltage dependence of channel activation. This is in contrast with a previous report that acetazolamide caused a small (−4.9 ± 1.4mV) hyperpolarizing shift

Table 2. Effects of 10μM Acetazolamide on Wild-Type α_{1A} Ca channels

Parameter (U)	Control (2mM Ba^{2+})			10μM Acetazolamide		
	Mean	SE (±)	n	Mean	SE (±)	n
Direct acetazolamide perfusion (pH-independent)						
Peak Current (pA)	−599	146	10	−572	133	10
V_{50act} (mV)	−13.7	1.9	11	−15.8	1.5	11
$V_{50inact}$ (mV)	−64.2	4.4	5	−66.1	2.8	5
τ_{act} (ms)	0.88	0.05	13	0.85	0.04	13
τ_{inact} (ms)	67.4	3.9	13	64.3	4.1	13
1–4 hour acetazolamide incubation (pH-dependent)						
V_{50act} (mV)	−12.7	1.4	8	−11.0	1.3	9
$V_{50inact}$ (mV)	−61.3	2.3	7	−60.14	2.1	8
τ_{act} (ms)	1.07	0.09	8	0.89	0.06	9
τ_{inact} (ms)	85.8	5.6	9	75.9	6.2	9

[a]Significant at $p < 0.05$.

SE = standard error.

in the voltage dependence of channel activation for wild-type channels.[13]

Previous studies in thalamic neurons have demonstrated that acetazolamide causes intracellular alkalinization in a nonbuffered system.[14] In a second experiment, we allowed cells expressing wild-type P/Q-type channels to incubate in a nonbuffered, Hepes-free media, containing acetazolamide (10μM) for 1 to 4 hours before patch clamping. Once again, we did not observe any changes in the biophysical properties of the wild-type P/Q-type currents.

It was hypothesized that acetazolamide would have an effect on the wild-type α_{1A} because the patient with this mutant improved clinically with acetazolamide treatment. When this was not observed, we explored the effect of acetazolamide on the H1736L mutant channel; however, the results we obtained (not shown) were identical to the wild type, demonstrating that acetazolamide does not affect the biophysical properties of either the wild-type or mutant P/Q-type Ca^{2+} channel.

There may be several explanations to account for our findings. It may be that our observations are limited by the exogenous expression of the P/Q-type channel in HEK cells and that these cells lack a critical modulatory component required for acetazolamide to affect channel properties. It also may be that acetazolamide acts via another mechanism not directly involving the P/Q-type Ca^{2+} channel.

In conclusion, we have identified a new EA2 mutation and have shown that, unlike previous missense mutations associated with EA2, the H1736L mutation does not completely block functional P/Q-type Ca^{2+} channel expression in a mammalian system. The new mutation may result in a loss of function through an overall reduction in inward mediated Ca^{2+}, although we also found evidence for some gain-of-function effects. It might have been expected that, because some patients with EA2 benefit from treatment with acetazolamide, this agent would have an affect on either the wild-type and/or mutated P/Q-type channels. In this study, we find that acetazolamide does not appear to directly affect the biophysical properties of either wild-type or mutant P/Q-type channels through either pH-independent or pH-dependent mechanisms.

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