Auxiliary subunits: essential components of the voltage-gated calcium channel complex

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Voltage-gated calcium channels are important mediators of several physiological processes, including neuronal excitability and muscle contraction. At the molecular level, the channels are composed of four subunits — the pore forming α_1 subunit and the auxiliary $\alpha_2\delta$, β and γ subunits. The auxiliary subunits modulate the trafficking and the biophysical properties of the α_1 subunit. In the past several years there has been an acceleration of our understanding of the auxiliary subunits, primarily because of their molecular characterization and the availability of spontaneous and targeted mouse mutants. These studies have revealed the crucial role of the subunits in the functional effects that are mediated by voltage-gated calcium channels.

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Abbreviations

AID	alpha interaction domain
AMPA	alpha amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
BID	beta interaction domain
EC	excitation–contraction
PDZ	PSD-95/disc large/zona occludens

Introduction

Voltage-gated calcium channels are multi-subunit membrane complexes that allow depolarization induced calcium influx into cells [1]. Voltage-gated calcium channels function in excitation–contraction (EC) coupling, excitation-secretion coupling, neurotransmitter release, regulation of gene expression and neuronal migration. Two classes of voltage-gated calcium channels have been described. The first class are high-voltage-gated channels, which are activated by strong depolarization. These are further classified into the P/Q, N, R and L types on the basis of differential biophysical properties and sensitivity to pharmacological agents. Relatively lower depolarization is sufficient to activate the second class of channels, which are known as the T-type channels.

Biochemical purification has revealed that high-voltage-gated calcium channels are composed of four subunits, including α_1, $\alpha_2\delta$, β and γ [1,2••]. The α_1 subunit forms the pore of the calcium channel. Both spontaneous mutations and targeted deletions of murine α_1 subunits have been identified or generated. It is now clear that mutations in these genes underlie some human diseases including episodic ataxia type 2, stationary congenital night blindness, familial hemiplegic migraine and spinocerebellar ataxia type 6. It has also been possible to identify proteins that are associated with different calcium channel complexes in different tissues [3]. However, only three 'auxiliary' subunits, $\alpha_2\delta$, β and γ that meet the following criteria have been identified. The criteria are (1) existence in purified channel complexes (2) direct interaction with the α_1 pore forming subunit (3) capability to directly modulate the biophysical properties and/or trafficking of the α_1 subunits and (4) stable association with the α_1 subunit.

In this review, we discuss the structural and functional diversity of the auxiliary subunits, spontaneous mutants and targeted mouse models of auxiliary subunits and their implications for human disease.

$\alpha_2\delta$ subunits

Four genetically distinct $\alpha_2\delta$ subunits $\alpha_2\delta$-1 – $\alpha_2\delta$-4, have been described [4–6]. Each one of these proteins is differentially expressed in various tissues, including skeletal muscle, heart and brain (Table 1). The diversity of each $\alpha_2\delta$ subunit arises by alternative splicing. At the protein level, all four subunits show conserved glycosylation sites, cysteine residues and predicted hydrophobicity profiles.

Of all the $\alpha_2\delta$ subunits, $\alpha_2\delta$-1 is the most extensively characterized. $\alpha_2\delta$ is a product of a single gene that is post-translationally cleaved into α_2 and δ peptides, which are then linked by disulfide bridges. The mechanisms that underlie the proteolytic cleavage and the disulfide linkage remain unclear. Topological analysis supports a model for the protein in which α_2 is entirely extracellular and δ has a single transmembrane region with a very short intracellular portion, which serves to anchor the protein in the plasma membrane (Figure 1; [7]).

α_2 is extensively glycosylated, a post-translational modification important in maintaining the stability of the

Table 1

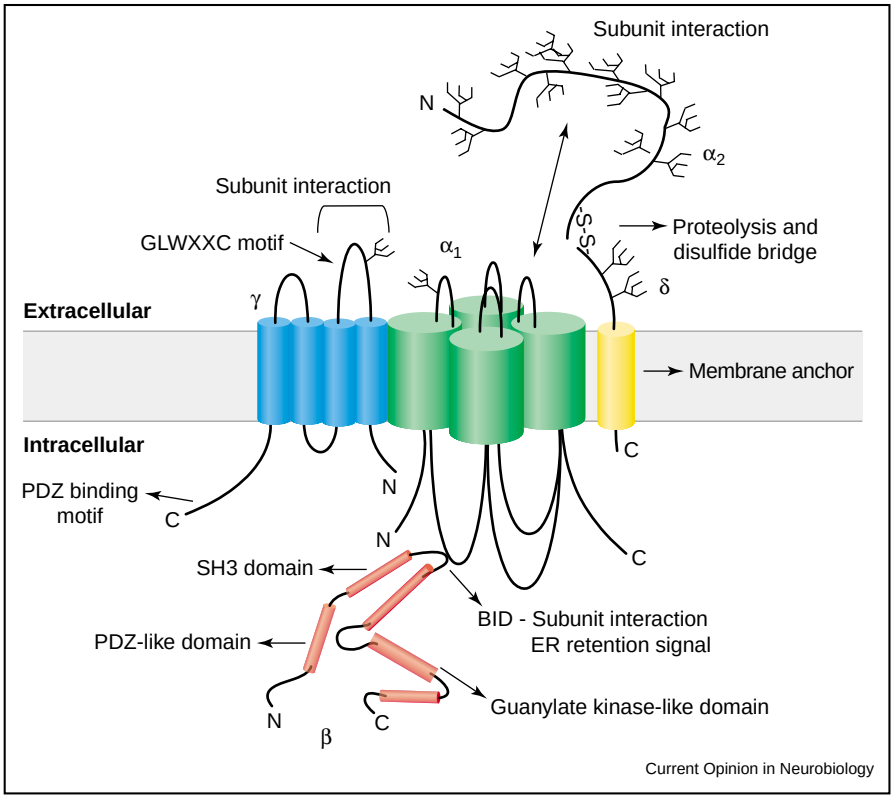
Chromosomal location, functional effects and tissue distribution of the auxiliary subunits of the voltage-gated calcium channels.

Subunit	Human chromosomal location	Functional effects	Tissue distribution	References
$\alpha_2\delta$-1	7q21-q22	Membrane trafficking of α_1 Increase in current amplitude activation/inactivation kinetics Voltage dependence of activation	Brain, heart, skeletal muscle	[7,9]
$\alpha_2\delta$-2	3p21.3	Increase in current amplitude	Lung, testis, brain, heart, pancreas, prostrate, skeletal muscle, spinal cord	[4,5]
$\alpha_2\delta$-3	3p21.1	Increase in current density Voltage dependence of activation Steady state inactivation	Brain, heart, skeletal muscle	[4]
$\alpha_2\delta$-4	12p13.3	Increase in current amplitude	Heart, skeletal muscle, intestine, fetal liver erythroblasts, adrenal gland, pituitary	[6]
β_1	17q21-q22	Skeletal excitation–contraction coupling Membrane trafficking of α_1 Targeting of $\alpha_1$1.1 to triads Increase in current amplitude activation/inactivation kinetics	β_{1a}-skeletal muscle, brain (other isoforms)	[16,23]
β_2	10p12	Increase in current amplitude activation/inactivation kinetics Targeting of $\alpha_1$1.4 in retina Membrane trafficking of α_1	Heart, lung, trachea, aorta, brain	[15,29]
β_3	12q13	Increase in current amplitude activation/inactivation kinetics Membrane trafficking of α_1	Smooth muscle, trachea, aorta, lung, brain	[27,30,31]
β_4	2q22-q23	Increase in current amplitude Activation/inactivation kinetics Membrane trafficking of α_1	Brain	[27,32]
γ_1	17q24	Inhibitory effect Activation/inactivation kinetics	Skeletal muscle	[33]
γ_2	22	Inhibitory effect Activation/inactivation kinetics Trafficking of AMPA receptor	Brain	[34–37,38•]
γ_3	16p13.1-p12	Activation/inactivation kinetics	Brain	[35–37]
γ_4	17q24	Inactivation kinetics	Heart, lung, brain, prostate, spinal cord	[35–37]
γ_5	17q24	?	Brain	[35]
γ_6	19q13.4	Reduction of current amplitude (low voltage-gated calcium channel)	Heart, skeletal muscle, brain	[52]
γ_7	19q13.4	Reduction of current amplitude	Brain, heart, lung, testis	[40•]
γ_8	19q13.4	?	Brain, testis, spinal cord	[35,36]

interaction with α_1 and is a major determinant of the protein's ability to stimulate the current amplitude [7]. There are forms of $\alpha_2\delta$ that are differentially glycosylated, however, the physiological consequences of this remain unclear. It is apparent that glycosylation of proteins has important functional consequences, and genetic defects in the pathways that enable glycosylation underlie some human diseases [8]. Given the widespread tissue distribution of $\alpha_2\delta$, the important physiological role, and the extensive post-translational modifications, it might be worthwhile to dissect out pathways that allow these post-translational modifications.

Interestingly, although δ is the portion of the protein that is anchored in the membrane, it is α_2 that interacts with the α_1 subunit [9]. This interaction is entirely extracellular, as the substitution of δ with an unrelated transmembrane domain does not prevent the interaction. The corresponding region on α_1 that interacts with $\alpha_2\delta$ is in the 3rd transmembrane domain. However, this domain is not sufficient to mediate interaction, which suggests that secondary interaction sites may exist. There is no evidence for the interaction of $\alpha_2\delta$ and β subunits and such interaction appears to be unlikely given the entirely cytosolic localization of β and the small region of $\alpha_2\delta$

Figure 1



Predicted membrane topology, subunit interactions and structural domains of the auxiliary subunits of the voltage-gated calcium channels. The voltage-gated calcium channels are composed of the pore forming α_1 subunit and the auxiliary $\alpha_2\delta$, β and γ subunits. The $\alpha_2\delta$ and γ subunits contain transmembrane domains, whereas the β subunit is entirely intracellular. All three auxiliary subunits interact directly with the α_1 subunit, with no known inter-auxiliary subunit interactions. Each of the auxiliary subunits contains unique structural domains as shown.

that is intracellular. In addition, there is currently no evidence for direct subunit interaction between the $\alpha_2\delta$ and γ subunits.

Functional effects

The co-expression of $\alpha_2\delta$-1 allows an enhancement in the membrane trafficking of α_1, associated with an increase in the number of ligand binding sites [10]. In addition, coexpression of the $\alpha_2\delta$-1 subunit causes an increase in current amplitude, faster activation and inactivation kinetics and a hyperpolarizing shift in the voltage dependence of activation. Some of these effects can be observed in the absence of the β subunit, whereas in other cases, the co-expression of β is required.

Interestingly, unlike $\alpha_2\delta$-1, the $\alpha_2\delta$-2 subunit only appears to increase the current amplitude, with no significant changes in the biophysical properties [5] of channels composed of $\alpha_1$1.2 or $\alpha_1$2.2.

In the case of co-expression of the $\alpha_2\delta$-3 subunit with $\alpha_1$1.3, the effects are more noticeable with co-expression of β. When $\alpha_2\delta$-3 was co-expressed with the $\alpha_1$1.3 subunit, the current density, time course of inactivation, and voltage dependence of steady state inactivation were unaffected [4]. There was, however, a slight shift in the activation curve in the hyperpolarizing direction. In contrast, when $\alpha_2\delta$-3 was co-expressed with β, there was a significant increase in the current density, a hyperpolarizing shift in the voltage dependence of current activation, and a shift of the inactivation curve in the hyperpolarizing direction. Similarly, $\alpha_2\delta$-3 shifts the steady state activation and inactivation curves of $\alpha_1$2.3/β_3 channels in the hyperpolarizing direction [7]. Studies with $\alpha_2\delta$-1 suggest that the increase of the current amplitude requires the presence of an intact α_2 subunit, whereas the modulation of the other biophysical properties requires the δ subunit [11].

$\alpha_2\delta$-4 is the most recently described subunit [6] and the least understood. Similar to the other $\alpha_2\delta$ subunits, $\alpha_2\delta$-4 enhances the currents generated by the $\alpha_1$2.1/β_3 subunits. The effect of $\alpha_2\delta$-4 on the other biophysical properties of the currents and the ability to modulate other α_1 subunits remains to be determined.

Although it is clear that the $\alpha_2\delta$ subunits are components of the high-voltage-gated calcium channel complexes, the native channels that they are associated with remain to be resolved. $\alpha_2\delta$-1 is the only $\alpha_2\delta$ subunit that has been extensively characterized by biochemical analysis. The availability of specific antibodies to each of the $\alpha_2\delta$ subunits and channel purification techniques will aid in clarifying native channel composition. The $\alpha_2\delta$ subunit is known to bind gabapentin, a widely used anticonvulsant drug, suggesting that it might be an important target for therapeutic intervention.

Mouse models

The ducky (du/du) mouse

The *du/du* mice have a genomic rearrangement that results in a truncated transcript for the $\alpha_2\delta$-2 gene and smaller protein product of α_2, with a complete loss of δ [12,13••]. Consequently, the protein is not anchored in the plasma membrane and appears to be entirely intracellular in transfected cells. Functionally, this truncated protein cannot compensate for the loss of the full-length protein. Electrophysiological studies reveal a severe reduction in the P-type current density in *du/du* mice with no change in the single channel conductance. It is not clear if this is caused by of loss of function or if the aberrant protein interferes with the complex formation or trafficking of the channel.

The ducky mice are smaller in size than their corresponding wild type littermates and fail to survive beyond about five weeks. The mice are characterized by a loss of balance and coordination (ataxia) and brief attacks of abnormal involuntary movement or posture (paroxysmal dyskinesia) and exhibit synchronous spike wave discharges, accompanied by behavioral arrest and response to ethosuximide (an anticonvulsant drug that acts on the brain and nervous system in the treatment of epilepsy). At the cellular level, Purkinje neurons in the ducky mice appear to not be completely developed, smaller and possess incomplete branching [12,13••].

Mice with targeted deletions or spontaneous mutations in $\alpha_2\delta$-1, $\alpha_2\delta$-3 and $\alpha_2\delta$-4 have not yet been described. In the future, it would be interesting to generate such mice to assess the specific roles of each of the $\alpha_2\delta$ subunits. These studies may include the role of $\alpha_2\delta$ in skeletal and cardiac EC coupling, independent of its role in the stimulation of the voltage-gated calcium currents. Furthermore, as it appears in cell expression studies that the $\alpha_2\delta$ subunits might be functionally heterogeneous, it would be interesting to assess if the loss of one of the subunits might lead to functional compensation by the others. The widespread expression of the $\alpha_2\delta$ subunits and their role in the modulation of calcium channels suggests that the loss of the protein might lead to severe physiological consequences.

β subunits

Four distinct genes encode the β subunits (β_1–β_4) and numerous splice variants are known [14]. All four of the genes are expressed in the brain. A distinct isoform of the β_1 subunit, the β_{1a} isoform, is a component of the skeletal muscle voltage-gated calcium channel. In addition to their expression in the brain, each β subunit shows differential expression in other tissues (Table 1).

β is the only subunit of the channel that is entirely cytosolic. Some forms, however, including β_{1b} and rat β_{2a} isoforms, can associate with the plasma membrane independent of the α_1 subunit. This is mediated by the presence of acidic motifs in the protein [15] or partly by lipid modification [16]. The ability of the rat β_{2a} to be inserted in the membrane is unique among the β_{2a} isoforms and is mediated by two amino-terminal cysteine residues that are palmitoylated and allow membrane insertion of the protein.

β subunits have a general structure comprised of five different domains, with the two central domains sharing significant homology amongst the β subunits [17]. The amino and carboxy termini are relatively less well conserved. Modeling studies predict the existence of at least three domains in the protein, namely a PDZ-like domain (PSD-95/disc large/zona occludens), an SH3 domain and a guanylate kinase-like domain. The physiological significance of the different domains and the distinct protein–protein interactions that they mediate is only just beginning to be understood.

β subunits associate with the α_1 subunit predominantly through a highly conserved high affinity interaction that is mediated by the Alpha Interaction Domain (AID) in the α_1 subunit [18] and a corresponding Beta Interaction Domain (BID) in the β subunit [19]. A mutation in the AID severely affects membrane trafficking and the ability of β to bind to α_1. In addition to the high affinity AID/BID interaction sites, secondary interaction sites have been described for β_4 and β_3. The presence of the conserved interaction domains on α_1 and β subunits allows for diversity of their interaction both *in vivo* and *in vitro*. This is reflected in the heterogeneity of β subunits that are associated with different channel complexes [20]. However, despite the ability of β subunits to form heterogeneous complexes, it is clear that each type of channel has a predominant β subunit associated with it. β_4 is the predominant subunit associated with the P/Q-type channels, whereas the N-type channels predominantly contain β_3.

Functional effects

The β subunit aids in the trafficking of α_1 to the plasma membrane, partly by its ability to mask an endoplasmic reticulum retention signal in the α_1 subunit [21]. In addition to its role in membrane trafficking, the β subunit

modulates the biophysical properties of the channel with characteristics specific to the $\alpha_1-\beta$ combination [22]. The β subunit can accomplish these dual functions independently, as illustrated by its ability to modulate the biophysical properties of channels in the presence of a mutation in the AID region, which disrupts its ability to enhance membrane trafficking of α_1. The mechanism for these independent functions of the same subunit is not clear, and it has been suggested to be a function of the ability of some of the β subunits to associate with other intracellular loops of the channel through interactions that are weaker than those described for the AID/BID.

In addition to their general role in enhancing trafficking of α_1, some β subunits have additional unique functions. β_1 is necessary for the targeting of $\alpha_1 1.1$ to the triads [23]. In addition to its role in trafficking α_1, β_{1a} in skeletal muscle functions in EC coupling [24]. The carboxy-terminal of β_{1b} contains a motif that allows the protein to be targeted to the plasma membrane in the absence of α_1. The amino-terminal of β_{2a} allows the protein to be anchored at the membrane and reduce the channel inactivation of $\alpha_1 2.1$ channels [25]. The carboxy and amino termini of β_4 allow the localization of the protein to presynaptic sites [26]. There is also evidence that the β subunit may be involved in targeting of the channel complex to certain cellular locations [27].

Regulation of calcium channels also occurs through modification of the β subunits. β_{2a} is a substrate for protein kinase A, and phosphorylation of β_{2a} is important for the ability of protein kinase A to stimulate the currents generated by the $\alpha_1 1.2$ channels in mammalian expression systems and in cardiac myocytes. Recent studies also demonstrate a direct role for the β subunit in the modulation of the $\alpha_1 2.2$ channels through the mitogen-activated protein kinase (MAPK) pathway [28•]. It is clear that the functional effects of the β subunits, and hence the calcium channels, can be modulated in response to a variety of cellular stimuli. Stimulus induced modification of the auxiliary subunits may provide an additional level of modulation of intracellular communication mediated by the voltage-gated calcium channels.

Mouse models

β_1 null mice

The β_1 subunit is expressed in a wide variety of tissues. A specific isoform, β_{1a}, is expressed in skeletal muscle. β_1 null mice have been generated by conventional gene targeting, but homozygous null mice die at birth. The fetuses of these mice are unable to move, show reduced L-type currents, have greatly reduced amounts of $\alpha_1 1.1$ and show a reduction in muscle mass with structural abnormalities [23]. Further studies have revealed a lack of EC coupling in these mice, and revealed a direct role for β_1 in EC coupling. The early lethality of these mice has not permitted a close examination of the effects of the loss of β_1 in the brain. However, recently a mouse model has been generated that expresses β_1 in the skeletal muscle but not other tissues [29]. The expression of β_1 in skeletal muscle allows bypass of the perinatal lethal phenotype. This mouse should provide a good model system to examine the effects of the loss of β_1 in the brain.

β_2 null mice

Mice that are deficient in β_2 have been generated by conventional gene targeting [29]. Homozygous null mice are embryonic lethal, presumably because of cardiac defects, as β_2 is a component of the cardiac voltage-gated calcium channel. It is unclear if β_2 has a role in the cardiac EC coupling, similar to β_1 in skeletal muscle.

Mice that express β_2 in the cardiac tissue, which allows bypass of the early lethal phenotype, have been generated [29]. A close examination of these mice has revealed retinal abnormalities, comparable with that observed in human congenital stationary night blindness. The absence of β_2 also results in the distortion of the distribution of $\alpha_1 1.4$. The availability of this mouse model should allow finer dissection of the role of β_2 in the brain.

β_3 null mice

β_3 null mice have been generated by conventional gene targeting strategy [30,31]. These mice appear to be normal in appearance with no obvious phenotype. A closer histological examination of different brain regions has revealed no gross morphological changes. In addition, there are no detectable abnormalities in the heart, lung, kidney, spleen, pancreas, liver, ovary or testis. The loss of β_3 results in the reduction of the N- and L-type currents and an alteration of the kinetics of the P/Q-type currents in the superior cervical ganglia. In smooth muscle, the lack of β_3 does not diminish L-type currents or affect smooth muscle contractility [30]. However, on a high salt diet, mice that lack β_3 have elevated blood pressure, suggesting a role for the voltage-gated calcium channels in maintaining blood pressure.

Lethargic mice

The lethargic *(lh/lh)* mouse arose as a spontaneous mutation in the gene encoding the β_4 subunit. The mutation in the *lh/lh* mouse has been characterized as a 4 bp insertion [32] within a 5′ splice site. This causes exon skipping and the generation of transcripts with a premature stop codon. The predicted protein, if produced, would be severely truncated and lack the BID, the domain that allows interaction with α_1. Subsequent studies [32] have demonstrated that there is no detectable protein, indicating that this is indeed a null mutation.

Lethargic mice exhibit ataxia, lethargic behavior and spontaneous focal motor seizures. A second seizure type, brief episodes of behavioral immobility, accompanied by

generalized cortical spike wave discharges is also observed. These seizures resemble the absence seizures of human *petit mal* epilepsy. The mice survive anywhere from 15 days to 2 months, show a reduction in body weight, immunological problems and increased mortality. Mice that survive beyond two months recover much of their immune function and body weight but exhibit reduced fertility. The absence of β_4 does not result in any change in the P/Q-type currents in the Purkinje neurons [32]. The ability of β subunits to form heterogenous complexes with α_1 subunits is reflected in the compensation for the loss of β_4 in the lethargic mouse [32]. However, the compensation is not functionally complete, as the lh/lh mouse still has a severe phenotype, indicating that all functions that are associated with β_4 are not completely compensated for by the association of other β subunits with neuronal calcium channels. It is tempting to speculate that one of the reasons for the lack of compensation is due to the ability of β_4 to interact specifically with certain cellular proteins that allow specific downstream effects.

It would be interesting to examine the effect of the loss of β_2 and β_1 on neuronal excitability. Mice that lack β_3 and β_4 in the brain display different phenotypes, suggesting that β subunits are not functionally equivalent. In the future, it would be interesting to generate mice that lack two or more β subunits to understand the complete effects of the loss of β subunits in the nervous system.

γ subunits

γ was originally known to only be associated with the skeletal muscle voltage-gated channel complex [33]. However, recent studies with the stargazer mouse revealed the existence of a neuronal γ subunit [34]. Subsequently, several γ subunits have been identified [35–38•]. The γ_1 subunit is most closely related to the γ_6 subunit, while γ_2, γ_3, γ_4 and γ_8 share significant homology. The expression of the γ subunits shows wide tissue distribution (Table 1), but the γ_1 subunit expression is restricted to skeletal muscle. The γ_2 and γ_3 subunits are associated with the P/Q- and the N-type channels [2••,36]. The *in vivo* partners of the other γ subunits remain to be identified.

γ subunits share a conserved four transmembrane domain topology with predicted intracellular amino and carboxy termini. In addition, γ subunits share common features including a GLWXXC amino acid motif in the first extracellular loop and several conserved residues. Neuronal γ subunits also share a consensus site for cAMP/cGMP phosphorylation. Consensus sites for N-linked glycosylation sites are present in the first extracellular loop. The physiological consequences of glycosylation are not yet understood. It would be interesting to determine if glycosylation plays a role in stabilizing the interaction of γ with the voltage-gated calcium channels, similar to that observed for $\alpha_2\delta$. γ_2, γ_3, γ_4, γ_5, γ_7 and γ_8 subunits contain a PDZ binding or related motif at the carboxy terminus.

Interestingly, γ_1, γ_2, γ_3 and γ_4 can traffic to the plasma membrane in the absence of the other subunits of the calcium channel in transiently transfected mammalian cells [39]. However, this does not appear to be a universal feature of γ subunits, as γ_7 does not traffic to the plasma membrane independent of the other subunits [40•]. The functional significance of independent plasma membrane trafficking and the effect of this on the association of γ with the other subunits of the channels is not clear.

The interaction domain of the γ subunit that cooperates with the voltage-gated calcium channel has been identified to be in the first half of the γ subunit [41]. In the muscular dysgenesis (*mdg*) mouse, which lacks $\alpha_1$1.1, $\alpha_2\delta$ and γ_1 are not associated, indicating that the $\alpha_1$1.1 subunit is required for γ to associate with the voltage-gated calcium channels. This is confirmed by the ability of $\alpha_1$1.1 and γ_1 to associate in heterologous expression systems [41].

Unlike β subunits that can functionally associate with several different α_1 subunits, both *in vitro* and *in vivo*, γ subunits appear to be more limited in terms of their subunit interaction. The γ_2 subunit does not form a complex with the skeletal channel [41], unlike the γ_1 subunit, suggesting that γ subunits may be more restricted in terms of their ability to form heterogeneous complexes.

Functional effects

Interestingly, unlike $\alpha_2\delta$ and β, γ subunits do not affect the number of channels on the cell surface. In addition, the absence of γ_1 does not perturb interactions within the other subunits of the skeletal muscle voltage-gated calcium channels [41], suggesting that γ_1 is not required to maintain the integrity of the channel complex. It appears that γ_1 predominantly functions in modulating the biophysical properties of the channel and does not have a significant role in the trafficking of the calcium channels, unlike the $\alpha_2\delta$ and β subunits.

γ_1 and γ_2 subunits have an inhibitory effect on the calcium currents [2••] and alter the activation and inactivation kinetics of $\alpha_1$2.1 and $\alpha_1$2.2 type currents. γ_2 and γ_3 subunits slightly alter the activation and the inactivation kinetics of $\alpha_1$2.1 type currents. γ_4 alters inactivation kinetics of $\alpha_1$2.1 type currents [39], and γ_7 severely reduces currents generated by N-type channels [40•].

Mouse models

γ_1 *null mice*

Two different groups have generated mice that lack γ_1 by targeted deletion [42,43]. These mice are viable,

fertile and show no obvious phenotype. The other components of the skeletal muscle voltage-gated calcium channel are expressed at normal levels [42] and the channel is maintained as a complex [41]. Closer examination of the voltage-gated calcium currents in skeletal muscle revealed an increase in the current density of the L-type currents, deceleration of the inactivation, and a shift in the steady state inactivation to more positive potentials. However, despite these effects on the L-type channels, no effect is observed on skeletal EC coupling [43], suggesting that unlike β_{1a} and $\alpha_1 1.1$, γ_1 does not have a direct role in EC coupling in skeletal muscle.

Stargazer mouse

The stargazer mouse arose as a spontaneous mutation in the gene that encodes γ_2 [34]. The mutation arises because of a retrotransposon insertion in an intron, and results in the complete loss of any detectable protein [2••].

The stargazer mouse is characterized by distinctive head tossing, ataxia, spike wave seizures and behavioral arrest, all of which are characteristic of absence epilepsy in humans. Waggler, an allele of stargazer, has also been described. Similar to stargazer, waggler arose as a spontaneous mutation. The waggler mouse is characterized by abnormal gait and motor coordination, and impaired eyeblink conditioning. These mice also display ataxia, but a lower frequency of head tossing than stargazer.

At the molecular level, in addition to the total loss of γ_2, the stargazer mouse displays a specific and severe reduction in brain-derived neurotrophic factor (BDNF) in the cerebellum [44], but not in the levels of the TrkB receptor. However, there is a significant reduction in tyrosine phosphorylation of proteins involved in the downstream signaling pathway. There also appears to be a delay in the disappearance of the external granule cells in the cerebellar cortex. In addition, the mice display impaired eyeblink conditioning. The exact correlation between loss of γ_2, reduction of BDNF and the epileptic phenotype remain unclear. Loss of γ_2 also results in impaired trafficking of AMPA receptors to the surface of cerebellar granule cells [45]. In the waggler mice, cerebellar synapse maturation defects are observed, and there is a lack of AMPA receptor mediated currents at the mossy fibre–granule cell synapses. It is apparent that the loss of γ_2 leads to the resultant phenotype because of its effect on multiple pathways.

Mice that either lack or have spontaneous mutations in the γ_3–γ_8 subunits have not been described.

Interaction of auxiliary subunits with other proteins

A few proteins that interact with the auxiliary subunits have been identified.

Gem

Gem is a small Ras related G protein that has been demonstrated to bind with the β subunit [46••]. The protein also binds calcium/calmodulin and inhibits the trafficking of the α_1 subunit to the plasma membrane. The binding of the activated calcium/calmodulin to Gem allows a nucleotide exchange (from GDP to GTP). In the GTP bound form, Gem has a high affinity for the β subunit and binding of the GTP bound form of Gem to the β subunit interferes with its ability to traffic the α_1 subunit to the plasma membrane. Presumably, the GTP on Gem is then hydrolyzed, and Gem can then be further activated by calcium/calmodulin. Functionally, this interference with trafficking of the α_1 subunit can lead to a decrease in calcium dependent exocytosis. These protein–protein interactions allow finer regulation of physiological events that are modulated by the voltage-gated calcium channels. Interestingly, this protein is not detected in the brain, raising the possibility that other similar proteins might be involved in the regulation of neuronal voltage-gated calcium channels.

AMPA receptor and PSD95

In addition to its role as a calcium channel subunit, there is evidence that the γ_2 subunit interacts with other neuronal proteins, in particular the AMPA receptor subunits [45] and the proteins of the post synaptic density, PSD95, SAP97, PSD93 and SAP102 [45]. Different domains of the γ_2 subunit are involved in interacting with the AMPA receptor subunits and the post synaptic density subunit proteins. Functionally, γ_2 regulates the membrane trafficking of the AMPA receptor subunits through an interaction that does not require its PDZ binding domain. In addition, γ_2 allows the synaptic targeting of the AMPA receptors. This is mediated by its ability to interact with the post synaptic density proteins through the PDZ binding domain at the carboxy-terminal.

It is not clear if distinct parts of the γ_2 subunit interact with the calcium channel and the AMPA receptor/PSD proteins. Alternatively, it also possible that the AMPA receptors and the calcium channels are closely associated in a large complex, with the γ_2 subunit serving as the protein that links the two complexes. Further studies are necessary to clarify these associations. Indeed, the identification of γ_2 as a protein with dual functions, both as a calcium channel subunit and in the trafficking of the AMPA receptor, is exciting.

The auxiliary subunits had so far only been known to be involved in specifically modulating the properties and trafficking of the voltage-gated calcium channels. It is well known that diversity of subunit interaction is possible both *in vitro* and *in vivo*, thus allowing a combination of different responses mediated by the same channel. This also has implications for the specific interactions of the proteins that act together with the auxiliary subunits.

Considering the number of different genes that encode the auxiliary subunits, it is conceivable that the auxiliary subunits themselves mediate specific interactions with other proteins in the nervous system, which in turn affects the voltage-gated calcium currents and the events that are modulated by the calcium influx. Future studies to further identify specific proteins that interact with the auxiliary subunits would help to understand how these interactions contribute to subtle neuronal responses to physiological stimuli.

Implications for human disease

It is clear that mutations or deletions of most of the auxiliary subunits result in a discernable phenotype and a loss of normal function in mouse models (Table 2). This is not surprising, considering the important physiological role of the calcium channels.

Few human diseases that arise from mutations or functional compromise of the auxiliary subunits have been described. Of these, two mutations in the gene encoding β_4 have been identified as potential causes of familial epilepsy and ataxia [47]. Lambert Eaton Syndrome is an autoimmune disorder characterized by muscle weakness. Immune sera from these patients react with voltage-gated calcium channels. Interestingly, there is evidence that these patients also generate antibodies to β subunits. In some cases, the antibodies generated inhibit the interaction of the β subunit with α_1 [48]. $\alpha_2\delta$-2 was originally cloned in the search for a tumor suppressor gene. The gene is located on the human chromosome 3p21.3 at a region that is frequently deleted in several small cell lung cancers. Interestingly, the transcript for the gene is well expressed in the lung and the protein can be detected in some lung tumor cell lines. Further studies are necessary to evaluate if the loss of the $\alpha_2\delta$-2 is involved in the initiation or the progression of the lung cancers. It is interesting to note that in several cases with Lambert-Eaton syndrome, small cell lung carcinoma is observed.

Multiple Sclerosis is one of the most commonly observed neurological disorders, characterized by demyelination and axonal damage. Though the exact etiology remains unclear, there is some evidence for a genetic predisposition to the disease. Recent studies have indicated that one of the predisposing loci for this disease is localized on human chromosome 17q and includes the regions that encode the γ_1, γ_4 and γ_5 subunits of the voltage-gated calcium channels [49]. Further studies are necessary to evaluate if the γ subunits are indeed candidate genes for multiple sclerosis.

Andersen's syndrome is a rare disorder that arises sporadically or is genetically inherited. The gene has been linked to chromosome 17q23, and mutations within the gene that encodes the Kir2.1 potassium channel have been identified in some cases [50], although it is clear that the disease is genetically heterogeneous. Interestingly, this region also contains the gene encoding the γ_1 subunit. It would be interesting to assess the possibility that mutations in this gene are also involved in the disease.

The role of genetic factors in childhood absence epilepsy have been shown, however, the genes that underlie these factors remain unclear. Recent studies have indicated that the locus for this disease also includes the gene that

Table 2

Spontaneous mutations and targeted deletions of the auxiliary subunits in mice.

Subunit	Spontaneous mutation/ targeted deletion	Phenotype	References
$\alpha_2\delta$-1	None known	?	
$\alpha_2\delta$-2	Spontaneous — ducky *(du/du)*, allele of ducky - du^{2J}	Ataxia, paroxysmal dyskinesia, synchronous spike wave discharges, accompanied by behavioral arrest and response to ethosuximide.	[12,13••]
$\alpha_2\delta$-3	None known	?	
$\alpha_2\delta$-4	None known	?	
β_1	Targeted deletion	Die at birth, fetuses are unable to move, reduced muscle mass with structural abnormalities, lack of skeletal excitation contraction coupling, transgenic mice that express β_1 in the skeletal muscle appear normal.	[23,24]
β_2	Targeted deletion	Embryonic lethal, retinal abnormalities in transgenic mice that express β_2 in the cardiac tissue.	[29]
β_3	Targeted deletion	Normal in appearance with no obvious phenotype, no gross morphological changes in brain or other tissues, elevated blood pressure on a high salt diet.	[30,31]
β_4	Spontaneous – lethargic *(lh/lh)*	Ataxia, lethargic behavior, spontaneous focal motor seizures, brief episodes of behavioral immobility, accompanied by generalized cortical spike wave discharges.	[32]
γ_1	Targeted deletion	Viable, normal in appearance, no phenotypic abnormalities.	[42]
γ_2	Spontaneous — stargazer *(stg/stg)*, waggler	Head tossing, ataxia, spike wave seizures and behavioral arrest, impaired motor coordination	[34]
γ_3–γ_8	None known	N/A	

encodes the γ_3 subunit [51]. Further studies are necessary to evaluate the role of γ_3 in this disease.

From studies on spontaneous and targeted mouse models it is clear that loss or mutation in most of the auxiliary subunits can have severe consequences, further emphasizing the role of these proteins in maintaining normal neuronal and muscular function. This implies that defects in these proteins may be considered as potential candidates for the underlying causes of some human genetic disorders, including certain forms of epilepsies whose molecular origin is unknown.

Conclusions

The auxiliary subunits of voltage-gated calcium channels mediate important physiological functions and the loss or mutation of these subunits can have severe consequences. The availability of mouse mutants, both spontaneous and targeted, will help significantly to enhance our understanding of the physiological and pathophysiological roles of the auxiliary subunits. Further studies on these mouse models should help to elucidate the intricate role of the auxiliary subunits in neuronal communication. The genes encoding these subunits may be considered as potential candidates in the search for genes underlying several human genetic disorders, including ataxias, seizures, migraines and epilepsies.

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