Structural determinants of calcium channel β subunit function

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Summary

L-, N- and P-/Q-type voltage-dependent calcium channels share a common structure, and are composed of at least one primary α_1 subunit and auxiliary β and $\alpha_2\delta$ subunits. Expression of the α_1 subunit alone in mammalian cells usually results in small calcium currents, and these are increased many times in amplitude by coexpression of auxiliary β and $\alpha_2\delta$ subunits. The β subunit, a cytoplasmically located protein, clearly regulates the calcium current through its interaction with the α_1 subunit, altering the latter's conformation and promoting its transportation to the plasma membrane. In the presence of β subunits in heterologous expression systems, calcium current amplitude is comparable to that of native channels; the kinetic properties and voltage-dependence of the channels also resemble those of native calcium channels.

Molecular properties of voltage-dependent calcium channels

Although calcium channels are expressed at very low densities in most tissues, in skeletal muscle they are enriched in the T-tubule system and serve as voltage sensors to trigger excitation–contraction coupling.[1,2] The skeletal muscle calcium channel (L-type channel) is sensitive to dihydropyridines and binds this class of drugs with high affinity. The abundance of calcium channels in skeletal muscle and their high affinity for dihydropyridine binding were essential for the purification of voltage-dependent calcium channels.[3–6] Data derived from the L-type calcium channels in the skeletal muscle have greatly facilitated our understanding of other types of calcium channels in cardiac and brain tissues.

The purified calcium channel from skeletal muscle is a complex containing five subunits (α_1, α_2, β, γ and δ) (fig. 1). The α_1 subunit is a 170 kDa protein which binds dihydropyridine drugs that either inhibit or stimulate calcium channel activity.[7–12] The protein contains extracellular, intracellular and hydrophobic domains but has not been reported to be glycosylated. The α_1 subunit is a substrate for protein kinase A, protein kinase G, protein kinase C, casein kinase II and calmodulin (CaM)-dependent protein kinase II.[13–15] The 140 kDa α_2 subunit is heavily glycosylated and is exclusively present in the extracellular space.[16] It is linked by disulphide bonds to the δ subunit, which contains a hydrophobic transmembrane domain. The β subunit, which is approximately 52 kDa in size and largely hydrophilic,[5,6] is a substrate for many kinases, including protein kinase A, protein kinase C and a protein kinase intrinsic to

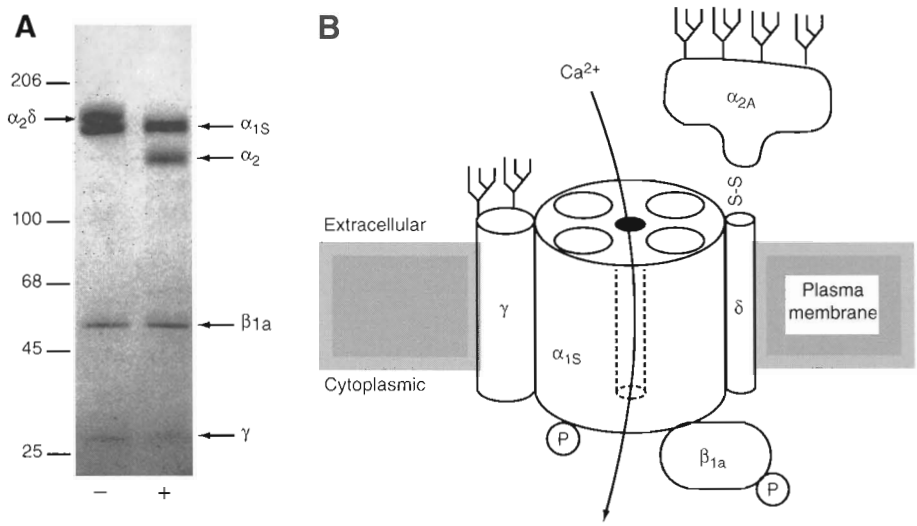

Fig. 1. A) Subunits of the purified skeletal muscle L-type calcium channel are shown separated on an SDS-polyacrylamide gel stained with Coomassie blue under non-reducing (–) and reducing (+) conditions.[13] The α_{2A} and δ subunits, which are linked by disulphide bonds under non-reducing conditions, separate into two proteins under reducing conditions. Molecular weight standards are shown on the left. B) Model of the skeletal muscle L-type calcium channel. The α_{1S}, γ and δ subunits are transmembrane proteins; the $\alpha_{2A}\delta$ and γ subunits are glycosylated; the β_{1a} subunit lies within the cytoplasm. P = phosphorylation sites.

skeletal muscle triads.[13–15] The γ subunit is uniquely expressed in skeletal muscle and also contains hydrophobic transmembrane domains.[17] All five subunits are tightly associated and copurify during the various stages of channel purification.

cDNAs coding for all five subunits have now been isolated. The amino acid sequence deduced from cDNA for the α_1 subunit predicts a protein with 24 transmembrane domains which group into four similar repeats.[18] The fourth hydrophobic domain of each repeat contains an orderly pattern of positive charges which could be the voltage sensor for the calcium channel. The structure of the α_1 subunit is comparable to that of the voltage-dependent sodium channel and, to a certain extent, that of the potassium channel.

The α_2 and δ subunits (referred to as the $\alpha_2\delta$ subunit) are encoded by one gene and are post-translationally cleaved to give rise to two proteins.[19] The δ subunit contains one presumed transmembrane domain, leaving only five amino acids in the C terminus which can be located intracellularly. Although the α_2 subunit contains two hydrophobic regions, it has recently been shown to be entirely extracellular.[20] The cDNA sequence for the γ subunit indicates that this subunit has four apparent membrane-spanning domains and a few possible glycosylation sites.[21] As for the β subunit, its cDNA sequence predicts a hydrophilic protein with no hydrophobic domains.[22] After identification of the genes coding for the subunits of the skeletal muscle L-type channel, homologous cDNA sequences to the α_1, $\alpha_2\delta$ and β subunits have been isolated from other tissues. Overall, six α_1 genes (α_{1S}, α_{1A}, α_{1B}, α_{1C}, α_{1D}

and α_{1E}), four β genes (β_1, β_2, β_3 and β_4) and one $\alpha_2\delta$ gene have been identified.[23–26] Several splice variants for each α_1, $\alpha_2\delta$ and β gene have been reported.[27–31]

Based on the electrophysiological, pharmacological and molecular properties of the calcium channels studied so far, calcium channels can be grouped into six types (T, L, N, P, Q and R type) (table I). The T-type channel is distinguished from the other types by its activation at relatively negative potentials, and for this reason it is also known as the low-voltage-activated (LVA) channel.[32–34] It is responsible for the rhythmic electrical activity in cardiac muscle and endocrine cells. All the other types of calcium channels are high voltage activated (HVA) (see table I). The L-type channel is uniquely sensitive to dihydropyridine drugs. Three α_1 subunits (α_{1S}, α_{1C} and α_{1D}) can independently form L-type channels and therefore they are grouped together as the L-type subfamily (see table I).[27,35–38] The α_{1C} subunit is expressed in cardiac muscle and brain, while the α_{1D} subunit is present in neurons and endocrine cells (table II). L-type calcium channels are involved in excitation–contraction coupling, excitation–secretion coupling and control of gene expression.

The N-type channel is specifically blocked by two peptide toxins found in fish-hunting snails: ω-CTx-GVIA and ω-CTx-MVIIA (see table I).[39] The α_{1B} subunit binds these toxins and confers basic properties such as voltage-dependent activation and ionic permeability. The P- and Q-type channels are sensitive to the peptide toxins ω-Aga-IVA and ω-CTx-MVIIC (see table I).[40–41] These two channels are probably formed by α_{1A} subunits,[42] which have several isoforms (see table II) and appear to be expressed in several different sizes in neurons.[43,44] The R-type channel is blocked by ω-Aga-IIIA and,[45] in common with N-type and P-/Q-type channels, is expressed in neuronal tissues (see table I). N-type and P-/Q-type channels are principally involved

Table I. Properties of different types of calcium channel

	Channel type					
	T	L	N	P	Q	R
Activation threshold	>–70 mV LVA	>–30 mV HVA	>–30 mV HVA	>–40 mV HVA	>–40 mV HVA	>–40 mV HVA
Inactivation time-course	Fast	Slow	Moderate	Very slow	Moderate	Fast
Primary tissue location	Cardiac Neuronal Endocrine	Skeletal Cardiac Neuronal Endocrine	Neuronal	Neuronal	Neuronal	Neuronal
Pharmacology		DHPs (agonists/ antagonists)	ω-CTx-GVIA ω-CTx-MVIIA ω-CTx-MVIIC ω-Aga-IIIA	ω-Aga-IVA ω-CTx-MVIIC ω-Aga-IIIA	ω-Aga-IVA ω-CTx-MVIIC ω-Aga-IIIA?	ω-Aga-IIIA
Function	Membrane excitability	E–C coupling E–S coupling	E–S coupling	E–S coupling	E–S coupling	E–S coupling
Pore-forming subunit		α_{1S} α_{1C} α_{1D}	α_{1B}	α_{1A}?	α_{1A}?	α_{1E}?

Abbreviations: DHPs = dihydropyridines; E–C = excitation–contraction; E–S = excitation–secretion; HVA = high-voltage-activated; LVA = low-voltage-activated.

Table II. Properties of cloned α_1 subunits

Gene	Splice products	Chromosomal localisation (human)	Channel type	Distribution	Drug sensitivity
α_{1S}	a,b	1q31–q32	L	Skeletal muscle	Dihydropyridines
α_{1C}	a,b,c,d	12p13.3	L	Cardiac muscle, brain, smooth muscle	Dihydropyridines
α_{1D}	a,b,c,d	3p14.3	L	Endocrine tissue, brain	Dihydropyridines ω-CTx-GVIA
α_{1A}	a,b,c,d	19p13.1–13.2	P/Q	Brain, heart	ω-CTx-MVIIC ω-Aga-IVA FTX
α_{1B}	a,b	9q34	N	Brain	ω-CTx-GVIA
α_{1E}	a,b,c	1q25–q31	R?	Brain	Ni^{2+}

Abbreviations: FTX = funnel web spider toxin.

in neurotransmitter release, and the R-type channel may have a similar function also.[46,47]

Biochemical characterisation of cardiac L-type, brain N-type and brain P-/Q-type channels suggest that most voltage-dependent calcium channels may have similar subunit composition: an α_1, $\alpha_2\delta$ and β subunit (see table II; table III).[10,48–52] The heterogeneity of calcium channels forms the molecular basis for their diversity of function and this heterogeneity is primarily related to the existence of distinct α_1 subunits (see table II).[24,27] The α_1 subunits contain within themselves the functions of voltage-sensing, voltage-dependent activation and inactivation, drug sensitivity and ion permeability. However, cellular expression of the α_1 subunit alone usually provides insignificant calcium current. Furthermore, the β and $\alpha_2\delta$ subunits, in addition to increasing greatly the amplitude of calcium currents, profoundly alter many properties of calcium channels when coexpressed with α_1 subunits (see table III).[24,53–55] It is clear now that different β subunits also contribute to the functional diversity of calcium channels.[52,56–58]

Table III. Properties of cloned auxiliary subunits

Gene	Splice products	Chromosomal localisation (human)	Channel type	Distribution	Phosphorylation sites
β_1	a,b,c,d	17q21–22	Isoform a: L Rest: N, P/Q, other	a: skeletal muscle Rest: brain, heart, skeletal muscle	PKA, PKC, CK2
β_2	a,b,c,d	ND	P/Q, N, other	Cardiac muscle, brain	PKA, PKC, CK2
β_3	a,b,c	12q13	N, P/Q, other	Brain, cardiac muscle	PKC, CK2
β_4	a,b	ND	P/Q, N, other	Brain	PKC, CK2
$\alpha_2\delta$	a,b,c,d,e	7q21–q22	L, N, P/Q, other	Brain, heart, skeletal muscle	
γ		17q24	Skeletal muscle L	Skeletal muscle	

Abbreviations: CK2 = casein kinase II; ND = not determined; PKA = protein kinase A; PKC = protein kinase C.

Regulation of calcium channel function by the β subunit

Various β subunit cDNAs have been identified in humans, rat, rabbit, mouse, *Xenopus* and *Drosophila*.[59] These cDNAs are all similar in two regions (fig. 2 (A)), suggesting largely conserved structure for all the β subunits. Four different genes have been found in rat, and these are probably present in other species as well.[22,31,54,55,60] In addition, multiple splice variants have also been identified for human β_1, human β_2, rabbit β_2 and human β_3 genes, and it is likely that they exist for other β genes.[23]

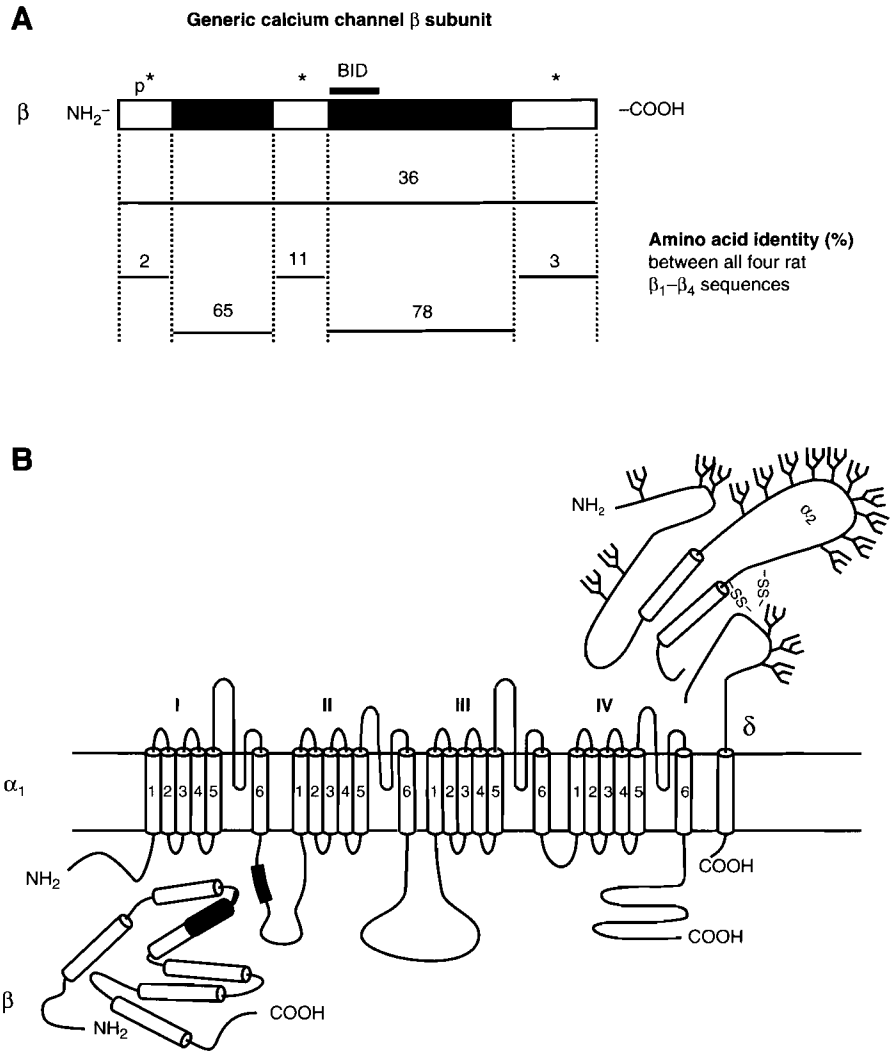

*Fig. 2. A) The generic β subunit of the calcium channel. Shaded regions represent highly homologous domains common to all four β subunits. The amino acid identities of different regions of the β subunits are obtained from a comparison of four rat β subunit sequences; * = involved in inactivation kinetics; BID (β interaction domain) = involved in binding to α_1 subunit, voltage-dependence, open probability, etc.; p = palmitoylation sites in β_{2a}. B) Schematic representation of the association between α_1 and β subunits of the calcium channel. Highlighted regions represent the α_1 interaction domain (AID; α_1 subunit) and β interaction domain (BID; β subunit). The α_1 subunit contains 24 transmembrane α-helical domains which can be grouped into four repeats.*

Notably, analysis of all β subunit sequences predicts many putative phosphorylation sites, including sites for protein kinase A, protein kinase C and casein kinase II, suggesting that the β subunit is a regulatory target for these protein kinases. Indeed, the β_{1a} subunit is phosphorylated *in vitro* by protein kinase A, CaM-dependent protein kinase II and protein kinase C.[13,15] Furthermore, β subunits contain an Src-homology-3 domain (SH3), based on sequence similarity to the known SH3 domains, suggesting a role for the β subunit in Ca^{2+}-mediated signalling and signal transduction.

Many combinations of α_1 and β subunits have been coexpressed in mammalian cell lines (COS-7, HEK293, L cell, etc.) and *Xenopus* oocytes. In all cases, β subunit coexpression increased the amplitude of the calcium current in comparison to α_1 subunit expression alone.[30,54,55,61] In most cases a 3- to 20-fold enhancement was recorded. In some studies, specific binding of calcium channel blockers was measured to determine whether β subunit coexpression increased the total number of calcium channels. β_1 subunit coexpression with the human α_{1B} subunit in HEK293 cells increased the number of binding sites for ω-CTx-GVIA without significantly affecting binding affinity,[62] and β_1 subunit coexpression with the rabbit α_{1C} subunit in Chinese hamster ovary (CHO) and COS-7 cells also increased the number of dihydropyridine binding sites.[63,64] However, α_1 protein level was not significantly altered by the presence of the β subunit in α_{1C} cotransfection experiments,[63] suggesting that the increase in current amplitude and channel binding sites may be related to cell surface localisation and a conformational change in the α_1 subunit in response to the β subunit, rather than to a change in α_1 protein synthesis. Indeed, β subunit coexpression altered the affinity of dihydropyridine binding to the α_1 subunit.[64]

The presence of the β subunit also modifies the channel kinetics of the α_1 subunit. The β subunit facilitates the activation kinetics of the α_{1S}, α_{1B} and α_{1C} subunits,[65,66] and slows the activation kinetics of the α_{1E} channel,[61] but has no effect on the activation kinetics of the α_{1A} subunit.[24] Interestingly, the most obvious difference among the various coexpressed β subunits is in their effects on the inactivation kinetics of the α_1 subunit.[56,57] In the case of the α_{1A} subunit, coexpression of the β_3 subunit induced the fastest inactivation kinetics, followed by the β_4 and β_{1b} subunits, whereas $\alpha_{1A}\beta_2$ channels showed rather slow inactivation. The inactivation time constants for the coexpressed $\alpha_{1A}\beta_2$ and $\alpha_{1A}\beta_3$ subunits can vary almost 10-fold. These results strongly suggest that the various β subunits can further broaden the functional phenotypes of calcium channels, enabling them to carry out diverse cellular activities.

β subunit coexpression has been shown to shift the voltage-dependence of activation of α_{1A}, α_{1C} and α_{1E} subunits to hyperpolarising potentials and to alter the voltage-dependence of inactivation of α_{1A}, α_{1B}, α_{1C} and α_{1E} subunits.[25,56,67] These functional modifications produced by the β subunit endow the expressed channel with properties resembling those of native calcium channels. In summary, the β subunit regulates the activity of the α_1 subunit in many ways, modifying the current amplitude, current kinetics and voltage-dependence of calcium channels.

Association of α_1 and β subunits of calcium channels

Given that the β subunit is an important regulator of α_1 subunit function, there are several important questions concerning the association of calcium channel α_1 and β subunits.

1. Are α_1 and β subunits associated in a 1:1 ratio?
2. Is there a specific assembly of one type of α_1 subunit with one type of β subunit in the cell?
3. Are there factors which can facilitate or disrupt α_1–β subunit interaction?

Several studies have addressed these questions. For the purified skeletal muscle L-type calcium channel, the stoichiometry of α_1, $\alpha_2\delta$, β and γ subunits was determined to be approximately 1:1:1:1.[68] The stoichiometry for the subunits in other types of calcium channels remains to be fully investigated. In addition, immunoprecipitation data suggest that virtually all skeletal muscle α_1 subunits, brain N-type channel α_1 subunits and P-/Q-type α_{1A} subunits are associated with β subunits.[52,58] However, it remains possible that some types of α_1 subunit may function *in vivo* in the absence of the β subunit. Six different α_1 genes and four distinct β genes have been identified, and the specificity of the α_1–β association has been investigated. It seems that most neurons express multiple types of calcium channels and several α_1 and β subunits.[69–72] In the rabbit brain, the α_{1B} subunit can be associated with any one of the β_{1b}, β_3 and β_4 subunits, and the α_{1A} subunit forms a complex with any one of the four brain β subunits (β_{1b}, β_2, β_3 and β_4).[52,58] In PC12 cells, the α_{1B} subunit of the N-type channel is associated with either the β_3 or β_2 subunit.[72] This association is mostly dependent upon the level of β subunit expression and, possibly, the affinity of the α_1–β subunit interaction. *In vitro* biochemical studies indicate that the α_1 and β subunits show high mutual affinity and that this interaction is not disrupted by high salt concentrations, small variations in physiological pH or the presence of detergents.[73] It remains to be seen if there are physiological factors which can affect this association.

Identification of a β subunit interaction site on the α_1 subunit

Several lines of evidence point to a direct interaction between the α_1 and β subunits of the calcium channel. First, calcium channel subunits are tightly associated in cells and remain associated during the purification process. Second, antibodies against α_1 or β subunits can co-immunoprecipitate the other subunit from native tissues. Third, the β subunit can regulate α_1 function when these two subunits alone are expressed. Finally, the *in vitro* synthesised β subunit has been shown to bind the purified α_{1S} subunit. Pragnell and colleagues have used a novel library screening method to identify the domains on the α_1 subunit responsible for β subunit interaction.[74] Radiolabelled β subunits synthesised *in vitro* were used to detect interactions with small fragments of α_1 subunit expressed from an α_1 epitope library. All positive clones thus identified contained a sequence of 18 amino acids localised at the cytoplasmic linker connecting repeats I and II of the calcium channel α_1 subunit. No other

interaction domains on the α_1 subunit have been identified by this method. The 18-amino acid binding region is termed the 'α_1 interaction domain' (AID), and mutations in this domain abolish the regulatory effects (current stimulation, shift of voltage-dependence of activation and changes in inactivation kinetics) produced by β subunit coexpression on the α_{1A} channel in *Xenopus* oocytes, demonstrating that the AID is necessary for β subunit attachment and regulation. This finding provides a molecular basis for β subunit function and suggests several possible ways in which the β subunit can modify the α_1 current. First, β subunit binding to the α_1 subunit via the I–II cytoplasmic linker may introduce conformation change in the α_1 subunit and thus alter some properties (voltage-dependence and open probability) of the calcium channel. Second, conformational change or β subunit association may protect the α_1 subunit from degradation. Third, while this interaction site anchors the β subunit to the α_1 subunit, additional interactions between these subunits may alter calcium channel function. Fourth, the β subunit may be required for the correct transport of the α_1 subunit to the surface membrane.

Conformational change in the α_1 subunit may account for some biophysical properties of the $\alpha_1\beta$ channel. In *Xenopus* oocytes, coexpression of the β subunit has been shown to increase the maximum conductance (g_{max}) and to shift the g_{max}–voltage curve towards a more hyperpolarising potential without affecting charge movement.[75] This suggests that β subunit association lowered the energy barrier required for the opening of the channel pore and increased the open probability of the channel. Results from transfection experiments with HEK293 cells differ from those obtained with oocytes in that the former show an increase in charge movement with β coexpression, albeit coupled with an increase in channel open probability.[76,77] In addition, binding studies with α_{1C} L-type channels expressed in CHO cells show increased dihydropyridine binding in the presence of the β subunit with no change in α_1 subunit expression.[63] Despite some discrepancies among these studies, overall this work suggests that the β subunit induces a conformational change in the α_1 subunit for ligand binding and channel gating.

Primary and secondary interactions between α_1 and β subunits

Shortly after the identification of the AID site, a β subunit region that interacts with the α_1 subunit was identified by expressing truncated β subunits with α_1 subunits in oocytes.[78] A 34-amino acid sequence of the β subunit, localised on the N terminal of the second conserved domain, maintained the ability to increase the current amplitude of the α_1 subunit. This sequence was later referred to as the 'β interaction domain' (BID) (see fig. 2 (A)), and mutations in the BID altered the ability of the β subunit to enhance the α_{1A} channel current. One mutation in the β_{1b} subunit (P221R) increased the α_{1A} current amplitude more effectively than did the wild-type β_{1b} subunit, while other mutations decreased the ability of the β_{1b} subunit to stimulate current through the α_{1A} channel. Although these mutations showed differences in their stimulation of the α_{1A} channel current, the α_1-interacting β mutations were similar in their abilities to shift the voltage-dependence of activation and

inactivation. These results suggest that the shift in voltage-dependence of activation and inactivation may result solely from the conformational change in the α_1 subunit after association with the β subunit, regardless of the nature of the β subunit. On the other hand, the kinetic properties of the calcium channel seem to depend on other regions of the β subunit besides the BID, because coexpression with the different truncations of the β subunit resulted in dramatic and irregular changes in current kinetics. Therefore it is possible that secondary interaction occurs between the β and α_1 subunits, with the BID acting as the primary interaction site with the AID (see fig. 2 (B)). Mutations disrupting the AID–BID association completely abolished β subunit regulation, suggesting that the primary interaction between these two molecules is a prerequisite for secondary interaction.

The N terminus of the β subunit has been demonstrated to control the inactivation kinetics of the α_{1E} subunit.[79] Thus, the β_1 subunit increased the inactivation of the α_{1E} subunit, while the β_2 subunit slowed α_{1E} inactivation. Chimeras produced from transposition of the N terminus of β_1 and β_2 subunits had opposite effects on α_{1E} subunit inactivation. This clearly indicates that the α_1 subunit interacts with the β subunit at a site outside the BID. Recently, the linker region between the two highly homologous domains of the β subunit has also been shown to regulate the rate of α_{1E} subunit inactivation.[80] In addition, the effect of the N terminus prevails over that of the linker region, which suggests that some modulation of the β subunit is required to unmask the effect of the linker domain. Thus, these results suggest that the β subunit interacts with the α_1 subunit at multiple sites in addition to the primary AID–BID binding site and that the inactivation kinetics may be determined by secondary interaction between α_1 and β subunits.

Localisation of the α_1 subunit and chaperone-like effects of the β subunit

Current stimulation, the most obvious effect of β subunit coexpression, may be partly attributable to the correct transport of the α_1 subunit to the plasma membrane.[81] Many proteins, including heterotrimeric G-protein α and γ subunits, are localised to the plasma membrane because of post-translational modification by the addition of lipid moieties such as myristoylation. The β subunit has such putative lipid modification sites. Although the β subunit is believed to be hydrophilic and cytoplasmic, the β_{2a} subunit has been shown to localise to the surface membrane when expressed alone in HEK (tsA201) cells. The β_{2a} subunit is post-translationally modified in HEK cells, and this modification results from palmitoylation (see fig. 2 (A)).[82] The protein level of the α_{1C} subunit remained similar with or without coexpression of the β_{2a} subunit. However, most of the α_{1C} subunit localised to the plasma membrane in the presence of the β_{2a} subunit, while immunostaining of the α_{1C} subunit transfected alone was largely perinuclear. Therefore, the β subunit is important for correct transposition of the α_{1C} subunit to the plasma membrane, through lipid modification and membrane localisation of the β subunit.

When mutations in the N-terminal cysteines of the β_{2a} subunit resulted in a palmitoylation-deficient β_{2a} mutant, this mutant β_{2a} subunit remained able to target the α_1 channel to the plasma membrane but failed to increase the macroscopic current.[82] This interesting observation suggests that targeting the channel alone may not account for the stimulation of channel current by the β subunit. Moreover, some unknown cytoskeletal interactions may play a role in targeting the β subunit to the plasma membrane.

Although it is unclear whether the calcium channel β subunit promotes the correct folding and modification of the α_1 subunit during its biosynthesis, this might be one of the mechanisms by which the β subunit increases cell surface expression of the α_1 subunit. Voltage-gated calcium channels are quite comparable to voltage-gated potassium channels: the calcium channel α_1 subunit is similar in structure to the potassium channel pore-forming α subunit, and the potassium channel α subunit is associated with a cytoplasmic protein, Kvβ1-3, which shares functional (but not structural) similarity with the calcium channel β subunit. Notably, Kvβ2 associates with the Kv1.2 potassium channel α subunit during an early stage of channel biosynthesis, and this association promotes cotranslational N-linked glycosylation of the Kv1.2 polypeptide and increases the stability of Kv1.2 protein.[83] Therefore it is possible that the calcium channel β subunit also plays a similar role to that of the potassium channel in stabilising the α_1 subunit and promoting the surface expression of the mature channel complex.

Interactions between calcium channel subunits and non-channel proteins

In vivo, α_1, β and other subunits of the neuronal calcium channel form a tightly associated complex which interacts with the synaptic vesicle docking proteins syntaxin, SNAP-25 and, possibly, other unidentified proteins.[84–86] Calcium channel activity is dynamically modified by protein kinases, heterotrimeric G proteins, ryanodine receptors, SNAP-25 and syntaxin.[87–94] The modulation of α_1 subunit function by β subunits may be altered by the presence of these other proteins. In fact, it is now recognised that modulation of calcium channels by G proteins is mediated by the Gβγ subunit rather than the Gα subunit, and that Gβγ subunits may interact with the calcium channel α_1 subunit at the site where the calcium channel β subunit is anchored.[95–98] This raises the possibility that G-protein-mediated inhibition of the calcium channel may allosterically modify the α_1–β subunit interaction. The close proximity of the Gβγ subunits and calcium channel α_1 and β subunits suggests a complex interaction between these proteins.

Conclusions

Calcium channels are involved in the pathogenesis of several human diseases, and calcium channels blockers are widely used to treat cardiovascular and neurological disorders.[99,100] To date, hypokalaemic periodic paralysis has been linked to the

mutations in the calcium channel α_{1S} subunit, and a number of diseases have been linked to the α_{1A} subunit gene, including familial hemiplegic migraine, episodic ataxia type 2 and spinocerebellar ataxia type 6.[101–104] In mouse models, tottering and leaner mice have been found to carry mutations in the α_{1A} subunit.[105] The diseases resulting from the α_{1A} subunit dysfunction, such as cerebellar abnormalities, all share similar phenotypes. More recently, the β_4 subunit gene has been linked to neurological disorders in lethargic mice,[106] which display ataxia and lethargic behaviour as well as seizures. These features are somewhat similar to the defects caused by α_{1A} subunit mutations. Notably, both α_{1A} and β_4 subunits are highly expressed in cerebellar Purkinje and granule cells.[24,57] The β_4 subunit has also been shown to be a dominant form of β subunit in α_{1A}-containing channels in brain tissue.[52] In addition, while the β_4 subunit is very important in α_{1A}-containing channels, it is also present in N-type and perhaps other types of calcium channels,[58] and is expressed in other brain regions. Therefore, absence of a functional β_4 subunit may impair several different types of calcium channel function. In skeletal muscle, β_1 subunit deficiency in the transgenic mouse is associated with a reduced calcium current and impaired excitation–contraction coupling.[107] Future research will probably identify more human disorders caused by calcium channel dysfunction. Since auxiliary subunits play an essential part in calcium channel function, a better understanding of the regulatory role of the auxiliary β subunit will be essential in understanding calcium channel dysfunction in disease.

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