Published in final edited form as:
Wiley Interdiscip Rev Membr Transp Signal. 2013 September 1; 2(5): 207–220. doi:10.1002/wmts.93.

Regulation of high-voltage-activated Ca^{2+} channel function, trafficking, and membrane stability by auxiliary subunits

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Abstract

Voltage-gated Ca^{2+} (Ca_V) channels mediate Ca^{2+} ions influx into cells in response to depolarization of the plasma membrane. They are responsible for initiation of excitation-contraction and excitation-secretion coupling, and the Ca^{2+} that enters cells through this pathway is also important in the regulation of protein phosphorylation, gene transcription, and many other intracellular events. Initial electrophysiological studies divided Ca_V channels into low-voltage-activated (LVA) and high-voltage-activated (HVA) channels. The HVA Ca_V channels were further subdivided into L, N, P/Q, and R-types which are oligomeric protein complexes composed of an ion-conducting $Ca_V\alpha_1$ subunit and auxiliary $Ca_V\alpha_2\delta$, $Ca_V\beta$, and $Ca_V\gamma$ subunits. The functional consequences of the auxiliary subunits include altered functional and pharmacological properties of the channels as well as increased current densities. The latter observation suggests an important role of the auxiliary subunits in membrane trafficking of the $Ca_V\alpha_1$ subunit. This includes the mechanisms by which Ca_V channels are targeted to the plasma membrane and to appropriate regions within a given cell. Likewise, the auxiliary subunits seem to participate in the mechanisms that remove Ca_V channels from the plasma membrane for recycling and/or degradation. Diverse studies have provided important clues to the molecular mechanisms involved in the regulation of Ca_V channels by the auxiliary subunits, and the roles that these proteins could possibly play in channel targeting and membrane Stabilization.

INTRODUCTION

Voltage-gated Ca^{2+} (Ca_V) channels are a family of transmembrane proteins widely distributed in excitable cells and also found in many nonexcitable cells. These channels open when the plasma membrane becomes depolarized mediating Ca^{2+} entry in response to action potentials and subthreshold depolarizing signals. Ca^{2+} entering the cell through Ca_V channels serves as the second messenger of electrical signaling, initiating a variety of cellular events including neurotransmitter and hormone release, muscle contraction, enzyme activation, and gene expression, among many others. Signal transduction in different cell

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Conflict of interest: The authors have declared no conflicts of interest for this article.

types involves diverse molecular subtypes of Ca_V channels, which mediate currents with distinct physiological and pharmacological properties.[1]

Ca_V channels have been grouped into two distinct classes depending on their voltage sensitivity (Table 1). Channels that are activated at relatively hyperpolarized voltage ranges (less than or equal to −40 mV) have been named low-voltage-activated (LVA) or T-type, whereas channels that are activated at potentials more positive to −40 mV have been termed high-voltage-activated (HVA). HVA Ca_V channels can be further subdivided into L-, N-, P-, Q-, and R-types (Table 1), by virtue of their distinct functional and pharmacological profiles.[1]

Members of the HVA class are heteromultimers of a pore-forming $Ca_V\alpha_1$ subunit that coassembles into a functional channel complex with auxiliary $Ca_V\beta$, $Ca_V\alpha_2\delta$, and in some cases with $Ca_V\gamma$ subunits (Figure 1(a)). In contrast, members of the LVA channel Ca_V class are thought to be $Ca_V\alpha_1$ subunit monomers.[2] The $Ca_V\alpha_1$ subunit defines the channel subtype, whereas the auxiliary subunits regulate the $Ca_V\alpha_1$ subunit function and plasma membrane expression.[1,3] Molecular studies have shown that the $Ca_V\alpha_1$ subunit is comprised of four homologous repeats each containing six transmembrane helices (S1–S6) and a pore lining P-loop domain (Figure 1(b)). These homologous membrane repeats are connected via cytoplasmic loops and flanked by intracellular N-and C-termini.[1]

Ten different $Ca_V\alpha_1$ subunits grouped into three subfamilies are expressed in mammals[1] (Table 1). The Ca_V1 subfamily encodes L-type Ca^{2+} channels and includes four members ($Ca_V1.1$–Ca_V 1.4). The Ca_V2 subfamily consist of three members ($Ca_V2.1$–$Ca_V2.3$) that encode the neuronal P/Q-type, N-type, and R-type channels, respectively. The P- and Q-type channels appear to arise from alternate splicing of $Ca_V2.1$ and/or coassembly with distinct $Ca_V\beta$ subunits.[4,5] Last, the Ca_V3 subfamily includes the LVA (T-type) channels with three members ($Ca_V3.1$–$Ca_V3.3$).

EFFECTS OF THE AUXILIARY SUBUNITS ON HVA Ca_V CHANNEL FUNCTION, TRAFFICKING, AND MEMBRANE STABILITY

Ca_V channel trafficking and regulation mechanisms involve many different aspects including intrinsic channel protein structural determinants, interactions with regulatory elements such as second messengers and/or with extrinsic proteins to the channel complex. However, as we shall discuss later, associations with auxiliary subunits $Ca_V\beta$ and $Ca_V\alpha_2\delta$ are thought to play crucial roles in fine-tuning channel gating, regulating channel modulation by other proteins and signaling molecules, as well as in forward trafficking and removal of Ca_V1 and Ca_V2 channels from the plasma membrane.[6,7]

THE $Ca_V\beta$ SUBUNITS

The $Ca_V\beta$ subunits are cytoplasmic proteins that bind to the proximal region of the intracellular loop between domains I and II of the $Ca_V1\alpha_1$ and $Ca_V2\alpha_1$ subunits termed the α-interaction domain (AID)[8] (Figure 1(b)). There are four subfamilies of $Ca_V\beta$ subunits ($Ca_V\beta_1$–$Ca_V\beta_4$), each with splice variants, encoded by four distinct genes.[9] All $Ca_V\beta$

subunits contain conserved Src homology 3 (SH3) and a guanylate kinase-like (GK) domains[9,10] (Figure 1(c)), placing them into the membrane-associated guanylate kinase (MAGUK) protein family, scaffold proteins that function at neuronal synapses, tight junctions, immunological synapses, and neutrophil membranes.[11] The general structural features of guanylate kinases are preserved in the GK domain of the $Ca_V\beta$ subunit (Cavβ-GK) though many key amino acid residues are absent rendering it catalytically inactive.[12] Instead, this domain has evolved into a protein–protein interaction module, binding tightly to the $Ca_V\alpha_1$ subunit through the AID.[13–15] Likewise, a large portion of the $Ca_V\beta$-GK domain remains free to interact with other proteins, such as RGK GTPases[16–18] and the largeconductance Ca^{2+}-dependent K^+ channels.[19] The SH3 and Cavβ-GK conserved domains are flanked by regions of variable sequence and length between isoforms and splice variants of each isoform.[9,12,20]

When coexpressed in heterologous systems with $Ca_V1\alpha_1$ or $Ca_V2\alpha_1$, $Ca_V\beta$ auxiliary subunits significantly enhance Ca^{2+} currents and affect the voltage dependence and kinetics of current activation and inactivation.[21–27] Furthermore, $Ca_V\beta$ subunit presence may be important for Ca_V1 and Ca_V2 channel modulation by different protein kinases,[28–32] G proteins,[33–35] Ras-related monomeric small GTP-binding proteins[36–39] as well as by proteins of the vesicle release machinery including Rab3-interacting molecule 1.[40–43] Results from $Ca_V\beta$ subunit knockout mice have confirmed the physiological relevance of these auxiliary subunits. $Ca_V\beta_1$ and $Ca_V\beta_2$ subunit knockouts are lethal,[44,45] while $Ca_V\beta_3$ and $Ca_V\beta_4$ knockouts result in severe malfunction of the central nervous system.[46,47]

Likewise, diverse studies have suggested that the $Ca_V\beta$ subunits may participate in physiological events independent of their association with Ca_V channels. It has been suggested that a truncated splice variant of $Ca_V\beta_4$ lacking the GK and the C-terminal domains interacts directly with the heterochromatin protein 1 (HP1), attenuating its function as gene silencing factor.[48] More recently, Zhang et al.[49] confirmed that full-length $Ca_V\beta$ subunits may function as transcription regulators. In particular, $Ca_V\beta_3$ can interact and suppress the transcriptional activity of Pax6(S), a critical factor for the development of the eye and the nervous system. In addition, upon neuronal differentiation, $Ca_V\beta_4$ can interact with B56δ, a nuclear regulatory subunit of the phosphatase 2A (PP2A), and the heterochromatin protein, HP1γ. This complex relocates to the nucleus, where it regulates the dephosphorylation of histones, a key mechanism in transcriptional regulation, and associates to the tyrosine hydroxylase (TH) promoter through the nuclear transcription factor thyroid hormone receptor α. This signaling cascade evidences the role of $Ca_V\beta_4$ as a repressor recruiting platform to control neuronal gene expression.[50,51]

Given their essential role in the functional expression and modulation of HVA Ca_V channels, it is not surprising that $Ca_V\beta$ auxiliary subunit mutations have been implicated in disease. Cavβ knockout animals and spontaneous occurring mutants have severe phenotypes that are in some cases lethal.[9,12,52] For instance, the $Ca_V\beta_{1a}$ subunit isoform is selectively expressed in skeletal muscle where it partners with Ca_V 1.1α_1 subunit and is necessary for excitation-contraction coupling. Consequently, the Cavβ_{1a} subunit knockout mice, similar to $Ca_V1.1\alpha_1$ knockouts, are motionless and die after birth from asphyxiation.[44] Similarly, $Ca_V\beta_{2b}$ expression is predominant in the heart and knockouts of this subunit die prenatally

due to lack of cardiac contractions.[45] $Ca_V\beta_3$ subunit knockouts show altered perception of inflammatory pain as a result of reduced N-type Ca_V channel expression in dorsal root ganglia[47] and have compromised sympathetic control, likely due to reduced N- and L-type channel activity.[53] Last, lethargic (*lh*) mice are naturally occurring $Ca_V\beta_4$ subunit knockouts. The lethargic phenotype includes ataxia, seizures, absence epilepsy, and paroxysmal dyskinesia,[46,52] associated with reduced excitatory neurotransmission in thalamic neurons.[54]

In humans, two point mutations in the $Ca_V\beta_{2b}$ subunit have been implicated in cardiovascular diseases. The first mutation contributes to a type of sudden death syndrome characterized by a short QT interval and an elevated ST-segment,[55] and the other is linked to the Brugada syndrome.[56] Likewise, mutations in the *CACNB4* gene have been linked to epilepsy and episodic ataxia.[57]

As mentioned earlier, $Ca_V1\alpha_1$ and $Ca_V2\alpha_1$ channel subunits show poor surface expression by themselves, however, upon coexpression of the auxiliary subunits currents are significantly increased reflecting either enhanced channel open probability or cell surface expression.[58,59] The increase in surface expression can be observed both in native and recombinant channels with any of the $Ca_V\beta$s and seems to be dependent on binding to the AID, as point mutations that disrupt the AID/$Ca_V\beta$ interaction reduce or abolish $Ca_V\beta$-mediated current stimulation.[8] Interestingly, in heterologous systems the GK domain can recapitulate the function of the full-length $Ca_V\beta$ subunits increasing channel surface expression.[60,61]

An initial explanation of how the $Ca_V\beta$ subunits enhance channel surface expression was that they antagonize an endoplasmic reticulum (ER) retention signal located in the I-II linker that severely restricts the plasma membrane incorporation of the $Ca_V\alpha_1$ subunit[58] (Figure 2(a)). More recently, this idea has been enriched based on data obtained after transferring the intracellular linkers of the $Ca_V1.2\alpha_1$ subunit into a T-type channel ($Ca_V3.1$). The results of these experiments suggest that the I-II linker of $Ca_V1.2\alpha_1$ has an ER export signal of 9 amino acids downstream the AID.[62] On the other hand, all other intracellular linkers, including the N- and C-termini, were found to contain ER retention signals. Consequently, it was proposed that the intracellular regions in the $Ca_V\alpha_1$ subunit form a complex that yields a prevailing ER retention signal, and when the $Ca_V\beta$ subunit binds to the I-II linker, it triggers a switch in the channel complex such that the ER export signal becomes dominant, enhancing $Ca_V\alpha_1$ surface expression (Figure 2(b)). In this process, the $Ca_V\alpha_1$ C-terminus may play also an important role for Cavβ-dependent channel upregulation.[12,62,63]

There are also studies that point to the importance of intramolecular domains in the physiology of the $Ca_V\beta$ subunits. In particular, it has been reported that $Ca_V\beta_3$ contains two PEST-like sequences, potential signals for rapid protein degradation,[64] sensitive to the Ca^{2+}-dependent protease calpain. $Ca_V\beta_3$ mutants lacking the PEST sequences induce increased $Ca_V2.2$ current densities,[64] suggesting that $Ca_V\beta$ subunit proteolytic cleavage may be important for Ca^{2+} channel surface expression.

Likewise, the role of $Ca_V\beta$s as critical determinants of channel cell surface expression has been also evidenced by protein–protein interaction assays. Using a yeast two-hybrid screening approach, Béguin et al.[36] initially uncovered a link between regulation of Ca_V channel trafficking and signaling by small guanosine triphosphatases (GTPases). Using the $Ca_V\beta_3$ subunit as bait to screen a cDNA library these authors identified the Ras-like GTPase kir/Gem as an interacting partner. Interestingly, the binding of kir/Gem to $Ca_V\beta_3$ inhibited their assembly with the $Ca_V\alpha_1$ subunit, such that kir/Gem cotransfection drastically inhibited Ca_V channel functional expression.[36]

In line with this, using an extracellular epitope to probe for surface expression of the channels, subsequent studies showed a drastic reduction of fluorescent signal when channels and their subunits were coexpressed with the small GTPase RGK.[37,38] This led to the initial conclusion that the RGK proteins disrupt the $Ca_V\alpha_1/Ca_V\beta$ subunit association, thus retaining newly synthesized channels in the ER and reducing the number of functional channels in the plasma membrane. However, it has been shown more recently that the reduction in surface expression of Ca_V 1.2 channels by small GTPases (e.g. Rem), seems to occur by enhancing the dynamin-dependent endocytosis pathway rather than by a sequestration of the $Ca_V\beta$ subunits by the small GTPase.[16] Interestingly, restoring Ca_V1.2 surface expression by coexpressing a dominant negative dynamin mutant was not sufficient to restore current densities, suggesting that Rem has at least one other mode of action to inhibit currents, most likely an effect on channel open probability.[65,66]

Recent work from Colecraft's group offers new insight into the complex mode of RGK-mediated Ca_V channel inhibition. According to their proposal which is based on a customized mechanism at both the channel and GTPase level, distinct RGKs differentially use $Ca_V\beta$-binding-dependent and $Ca_V\alpha_1$-binding-dependent mechanisms to inhibit Ca_V1 and Ca_V2 channels.[17] In this scenario, Rem may inhibit Ca_V 1.2 channel using both $Ca_V\beta$-binding-dependent and independent mechanisms and binding to $Ca_V\beta$ is required for Rem-mediated decrease in Ca_V1.2 channel surface density and open probability. On the other hand, Rem associates directly with $Ca_V1.2\alpha_1$ to initiate β-binding-independent inhibition while inhibits Ca_V2.2 channels using a solely $Ca_V\beta$-binding-dependent mechanism.[17]

The $Ca_V\beta$ subunits have been also implicated in trafficking of Ca_V channels to specific subcellular regions. Vendel et al.[67] found that $Ca_V\beta_{4a}$, one of the two alternative splicing variants of the N-terminal A domain of the $Ca_V\beta_4$ auxiliary subunit,[68] may exert functions other than modulation of channel gating and trafficking by interacting with the microtubule-associated protein 1A (MAP1A) as well as synaptotagmin I, an important protein for presynaptic vesicle release. These interactions suggest that the $Ca_V\beta_{4a}$ subunit may act as a scaffolding element to facilitate coupling of Ca^{2+} signaling with neurotransmitter release. Since the $Cav\beta_{4a}$-synaptotagmin I interaction is disrupted by Ca^{2+},[67] it has been speculated that at basal Ca^{2+} levels $Ca_V\beta_{4a}$ may interact both with the $Ca_V2.1\alpha_1$ subunit and the vesicle protein to organize the machinery necessary for vesicle release, and that Ca^{2+} entry via Ca_V channels disrupts this interaction, thus releasing the vesicle to allow fusion with the plasma membrane.[43]

More recently, it has been found that different members of the RIM family, putative effectors of Rab3, may functionally link Ca_V channels to proteins in the active zone at the presynapsis which are related to the machinery for exocytosis[69] (Figure 3). Moreover, it has been reported that several proteins in the active zone including RIM1 modulate neuronal $Ca_V2.1$ channels through interactions with the $Ca_V\beta$ subunits. These interactions not only modify the inactivation properties of the channels and cause a sustained Ca^{2+} influx, but also anchor the neurotransmitter-containing vesicles to the channels in the vicinity.[40,41] Similar results have been reported for recombinant L-type channels (of the $Ca_V1.2$ and $Ca_V1.3$ class) in insulin-secreting cells.[42]

THE $Ca_V\alpha_2\delta$ SUBUNITS

As in the case of $Ca_V\beta$s, the ability of the $Ca_V\alpha_2\delta$ auxiliary subunits to promote membrane expression of HVA Ca_V channels is well established.[6,70] Four subtypes of $Ca_V\alpha_2\delta$ proteins encoded by four separate genes with several known splice variants have been described.[71,72] In general, the *Cacna2d* genes (coding the $Ca_V\alpha_2\delta$ subunits) are translated as precursor proteins that are proteolytically cleaved into a transmembrane δ region and an extracellular α_2 domain, that remain linked by a disulphide bond.[3,6,70] The extracellular α_2 region is heavily glycosylated and contains several functional domains, including a 178 amino acid von Willebrand factor-A (vWFA) domain similar to extracellular matrix-binding regions of integrins, a 5 amino acid metal ion-dependent adhesion site (MIDAS) motif and a poorly understood 92 amino acid Cache domain[6] (Figure 1(d)). The transmembrane domain of δ and some extracellular regions of α_2 are thought to associate with the $Ca_V\alpha_1$ subunit of HVA channels.[73]

Although the functional relevance of the $Ca_V\alpha_2\delta$ subunits is not fully understood, there is compelling evidence indicating a role in trafficking and also in the modulation of channel biophysical properties. Heterologous expression of $Ca_V\alpha_2\delta$ with various $Ca_V\alpha_1$ and $Ca_V\beta$ subunits results in increased current densities, as well as altered current kinetics and current–voltage relationships.[23,25,74–79] The increase in current density is generally explained by improved targeting of Ca_V channels to the plasma membrane[74,78] and significant reduction in the rate of entry of surface resident channels into degradation pathways.[80] Additionally, the $Ca_V\alpha_2\delta$ subunits have been involved in cell surface organization of Ca_V channels. It has been shown that $Ca_V\alpha_2\delta$-1 (and likely the other $Ca_V\alpha_2\delta$ subunits) not only partitions into lipid rafts itself but also mediates raft-partitioning of neuronal $Ca_V2.2$ channel complexes.[81–83]

Recently, a mechanism has been proposed for Ca_V channel localization to lipid rafts which challenges the conventional structural model of the $Ca_V\alpha_2\delta$ subunits. This suggests that $Ca_V\alpha_2\delta$ associates with the plasma membrane via a glycosylphosphatidylinositol (GPI) anchor in the δ domain.[84] In order to examine the role of membrane anchoring of the $Ca_V\alpha_2\delta$-1 subunit on its biochemical and functional properties, Dolphin and her colleagues generated a $Ca_V\alpha_2\delta$-1 truncated at the putative GPI-anchor ω-site, which removes the C-terminal hydrophobic domain ($\alpha_2\delta$-1 ΔC-term[85]). Unexpectedly, the $\alpha_2\delta$-1 ΔC-term protein was able to produce a significant increase of Ca^{2+} currents in cells expressing $Ca_V2.1/Ca_V\beta_1$ channels. Likewise, these authors did not observe any effect upon external application of

secreted $\alpha_2\delta$-1Δ C-term, which suggested that an intracellular interaction with other subunits may be needed for the complete functionality of the $Ca_V\alpha_2\delta$-1 subunit in the channel complex. Last, a large proportion of $\alpha_2\delta$-1 Δ C-term was secreted into the medium, but despite the lack of a C-terminal membrane anchor, the $\alpha_2\delta$-1Δ C-term proteins remained partially associated with the plasma membrane. It has been speculated that this interaction may occur via a noncovalent linkage formed during the maturation and trafficking of the protein.[85] Other studies, have also shown that the raft localization of $Ca_V\alpha_2\delta$ is preserved after replacement of the GPI anchoring motif,[83] which is consistent with the idea that the $Ca_V\alpha_2\delta$ subunits may reside in lipid rafts via protein–protein and/or specialized lipid–protein interactions.

As mentioned earlier, during biosynthesis the $Ca_V\alpha_2\delta$ subunits are generated as precursor proteins which undergo post-translational cleavage, oxidation, and glycosylation to yield mature proteins comprised of disulphide-linked α_2 and δ glycopolypeptides.[86–88] Although the precise functional role of the post-translational modifications of the $Ca_V\alpha_2\delta$ subunits has not yet been fully defined, it has been suggested that they could play a role in channel trafficking. Initial experiments by Gurnett et al.[75] showed that *N*-linked glycosylation play a critical role for surface expression of $Ca_V2.2$ channels since the Ca^{2+} current stimulation normally observed after $Ca_V\alpha_2\delta$ coexpression was virtually abolished by deglycosylation. Moreover, by combining electrophysiology with site-directed mutagenesis two *N*-glycosylation sites were found in the α_2 domain of the protein that are required for the auxiliary subunit-induced current stimulation. Given that the mutation of these sites prevented current stimulation without altering its kinetic properties, a regulation on the number of functional channels at the plasma membrane was suggested.[86]

In addition, alterations in the proteolytic cleavage that typically separates the extracellular α_2 from the transmembrane δ domain[89] may interfere with proper assembly and trafficking of the $Ca_V2.2$ channel complex. After mutation of the amino acids in the proteolytic site, $Ca_V\alpha_2\delta$ becomes insensitive to proteolytic cleavage and loses its ability to increase currents. These changes are not accompanied with alterations in the voltage dependence or kinetics of the channels, suggesting a reduction in the number of channels targeted to the plasma membrane.[87]

Disulfide bond formation seems to be also important for channel cell surface expression. Recent studies have shown that a pair of conserved cysteine residues at positions 404 and 1047, located in the vWFA region of α_2 and the extracellular domain of δ, respectively, form a single intermolecular disulfide bridge required for normal $\alpha_2\delta$−1 subunit function.[88] In these studies, it was shown that whereas there was a significant increase in current density through $Ca_V2.2\alpha_1/\beta_3$ channels upon cotransfection with wild-type $Ca_V\alpha_2\delta$−1, Cys404Ser, and Cys1047Ser mutations had little influence in current density, implying that formation of an intra-subunit disulfide bond between these residues is essential for $Ca_V\alpha_2\delta$-1 functional enhancement of currents, and perhaps for Ca_V channel trafficking to the plasma membrane.

Likewise, some insights into the role of the $Ca_V\alpha_2\delta$ auxiliary subunits *in vivo* come from a series of spontaneously occurring mutations in mice. These include two mutations in $Ca_V\alpha_2\delta$-2 (*ducky* and *entla*) that result in similar phenotypes characterized by ataxia,

paroxysmal dyskinesia, and spike wave seizures.[52,90,91] In humans, a mutation in $Ca_V\alpha_2\delta$-4 that introduces a premature stop codon that truncates one-third of the corresponding open reading frame presumably underlies a channelopathy leading to cone-rod dysfunction in the visual system.[92] More recently, a novel gene defect in *CACNA2D2* encoding the $Ca_V\alpha_2\delta$-2 subunit has been associated with early infantile epileptic encephalopathy a disease that usually manifest as severely impaired cognitive and motor development.[93] Last, mutations in the *CACNA2D1* gene might be associated with some forms of cardiac dysfunction, including the Brugada and long QT syndromes.[94,95]

Interestingly, it has been reported that experimental peripheral nerve injury results in increased levels of $Ca\alpha_2\delta$-1 mRNA in damaged dorsal root ganglion (DRG) sensory neurons, as well as a corresponding increase in protein levels in the neuronal cell bodies and at their presynaptic terminals in the spinal cord.[96–98] Furthermore, mice overexpressing the $Ca_V\alpha_2\delta$-1 subunit show a neuropathic phenotype of hyperalgesia and tactile allodynia in the absence of nerve injury,[99] suggesting that $Ca_V\alpha_2\delta$-1 is instrumental to the excitability of DRG neurons and the expression of neuropathic pain.

Evidence supporting the role of the $Ca_V\alpha_2\delta$ subunits in the promotion of surface expression of channels comes, in part, from the fact that $Ca_V\alpha_2\delta$ is the principal target for a group of small molecules with analgesic effects called gabapentinoids.[100] Although the mechanisms by which these drugs alleviate pain is not well understood, they seem to act intracellularly after chronic but not acute treatment.[79,101–105] Therefore, Vega-Hernandez and Felix[102] initially formulated the hypothesis that gabapentinoids could be impairing the ability of $Ca_V\alpha_2\delta$ to enhance the number of channels in the plasma membrane, via an effect on trafficking. Subsequent studies showed that indeed GBP acting chronically by displacing an endogenous ligand that is normally a positive modulator of $Ca_V\alpha_2\delta$, could be interfering with the function of the vWFA domain impairing Ca_V channel trafficking to the membrane[78,104] (Figure 4).

In addition, gabapentin has been shown to inhibit Rab11-dependent recycling of N-type Ca_V channels from the plasma membrane.[106] However, not only the trafficking and recycling of recombinant Ca_V channels appears to be impeded by gabapentin but also the enhancement of native N-type channel at the cell surface associated with chronic pain.[98] It is worth mentioning that endosomal trafficking may represent an important mechanism for maintaining proper plasma membrane expression of distinct native Ca_V channels,[107,108] though the role of $Ca_V\alpha_2\delta$ in this process, if any, is yet to be determined.

Last, although investigations regarding the role of the auxiliary subunits on subcellular distributions of Ca_V channels have primarily focused on the $Ca_V\beta$ subunits in neurons,[40,41] initial studies using L-type channels have revealed that the $Ca_V\alpha_2\delta$ subunits may also play a role in this process. Hence, it has been reported that the RalA GTPase regulates Ca_V channel activity, and acts as a chaperone for hormone secretory granules to L- and R-type channels on the plasma membrane, where these granules become tethered, in preparation for the formation of excitosome complexes with vesicle and plasma membrane SNARE proteins. Notably, this interaction occurs through a physical association between RalA and $Ca_V\alpha_2\delta$-1,[109] suggesting that the RalA/$Ca_V\alpha_2\delta$-1 complex likely engage and traffic

secretory granules to the plasma membrane-bound $Ca_V\alpha_1$ subunits to be able to then conduct Ca^{2+} through functional channels at precise sites where hormone release occurs.

ROLE OF THE AUXILIARY SUBUNITS ON HVA Ca_V CHANNEL REGULATED DEGRADATION

Recent studies have proposed that the $Ca_V\beta$ auxiliary subunits increase channel surface expression by preventing $Ca_V\alpha_1$ ubiquitination and proteasomal degradation.[63,110] Indeed, $Ca_V1.2$ channels are tonically ubiquitinated and the degree of ubiquitination seems to be increased in the absence of the $Ca_V\beta$ subunits by an ER associated ubiquitin ligase called RFP2. Hence, in the absence of $Ca_V\beta$, an interaction occurs between the channel and proteins related to the ER Associated Protein Degradation (ERAD) system. The end result of this is channel retrotranslocation and proteasomal degradation in the cytosol. On the other hand, in the absence of the $Ca_V\beta$ subunits, a proteasome inhibitor can rescue $Ca_V\alpha_1$ surface expression.[110]

These results suggest that the Cavβ subunits may be required to help $Ca_V\alpha_1$ (particularly of the $Ca_V1.2$ and $Ca_V2.2$ class) escape the ubiquitin proteasome system (UPS) degradation pathway.[63,110] Consistent with this, reduced levels of bulk $Ca_V2.2$ and Ub-$Ca_V2.2$ proteins are observed in the absence of $Ca_V\beta$ subunits.[111] In this regard, elements in the proximal C-terminus of a $Ca_V2.2\alpha_1$ alternative splice variant (e37b) predispose cloned and native channels to downregulation by the UPS.[111] Therefore, the question arises as to whether sequences unique to e37b may destabilize the $Ca_V2.2/Ca_V\beta$ subunit interaction leading to increased ubiquitination and degradation by the UPS.

In a similar manner, it has been suggested that the $Ca_V\alpha_2\delta$-1 subunit may play a role in stabilizing Ca_V channel functional expression once delivered to the plasma membrane. By examining the binding, internalization, and degradation kinetics of recombinant N-type ($Ca_V2.2\alpha_1\delta/Ca_V\beta_{1b}$) channels in the presence or the absence of $Ca_V\alpha_2/\delta$-1, Bernstein and Jones[80] proposed that the $Ca_V\alpha_2\delta$ subunits may act, at least in part, reducing the entry of surface resident Ca_V channels into degradative pathways. Computer modeling of trafficking data showed that $Ca_V\alpha_2\delta$ stabilizes N-type channels after their delivery to the cell surface through a significant reduction in the rate constants for internalization and degradation.[80] These kinetic changes help to explain the enhanced N-type channel expression levels and suggest that, in conjunction with forward trafficking effects (see the preceding section), the $Ca_V\alpha_2\delta$ subunits play a significant role in defining HVA Ca_V channel functional expression.

Last, it is well established that skeletal muscle L-type Ca^{2+} channels contain a $Ca_V\gamma_1$ subunit which includes four transmembrane helices.[1,3] It was subsequently shown that neurons express at least four homologues of $Ca_V\gamma_1$,[112] most notably $Ca_V\gamma_2$ also known as stargazin,[113,114] which in addition to regulate Ca_V channel function may also play a role in mediating AMPA receptor trafficking to the synapsis.[115] In general, the γ subunits appear to be inhibitory and their physiological relevance is emphasized by the epileptic phenotype in the stargazer mouse, a spontaneous mutant that lacks the $Ca_V\gamma_2$ subunit.[52,112,113,115]

Although it has been shown that $Ca_V\gamma_1$, $Ca_V\gamma_2$, and $Ca_V\gamma_7$ drastically reduce Ca^{2+} current density in heterologous expression systems,[116–119] it is worth noting that these effects may not be associated with altered channel trafficking but most likely occur as a result of changes in channel expression. Interestingly, the effect of the $Ca_V\gamma$ subunits resemble the inhibition of the $Ca_V2.2$ currents induced by the coexpression of truncated $Ca_V2.2$ constructs,[120] which occurs after the activation of PERK, a component of the unfolded protein response (UPR).[118,121] When UPR is initiated, an immediate consequence is the inhibition of protein biosynthesis through phosphorylation of the translation initiation factor eIF2α.[122]

PERSPECTIVES

Molecular and cellular biological studies have begun to explore the intriguing question of the spatial and temporal control of Ca_V channel density in the plasma membrane. These studies will surely be followed with more mechanistic analyses of the trafficking, targeting, recycling, and degradation, as well as their interactions with the cytoskeleton and regulation by cellular activity. Future studies must consider also other important variables such as distinct cell phenotypes, developmental, and cell cycle stages, and the dynamic arrangement of microdomains within a given cell. Therefore, the molecular characterization of the possible roles of Ca_V channel auxiliary subunits is only one of the crucial early steps towards understanding how different cells regulate membrane expression and organization of Ca_V channels for functional purposes. Undoubtedly, the current shortage of knowledge will be the motivation for intensive work in this field in the near future.

Acknowledgments

This work was supported in part by a grant (128707-Q) from The National Council for Science and Technology (Conacyt, Mexico) to RF and from NIH (1K99MH099405-01) to AA. A doctoral fellowship from Conacyt to ACR is also gratefully acknowledged.

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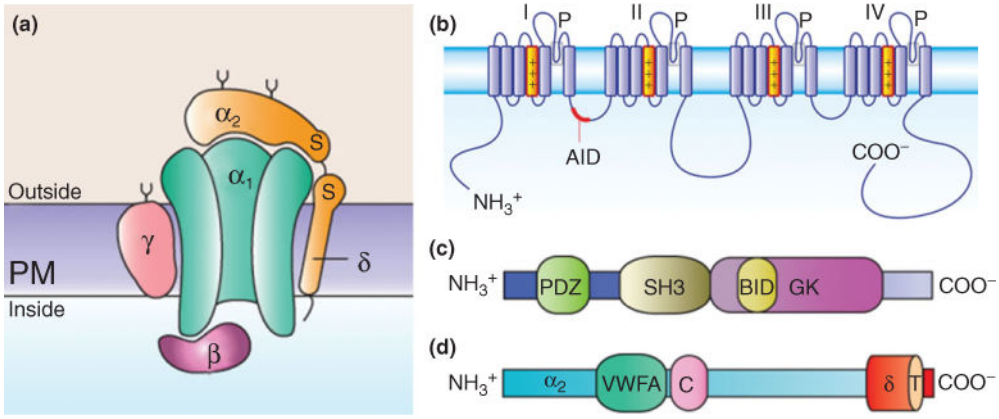

FIGURE 1.

Subunit composition of high-voltage-activated Ca^{2+} channels and structural domains of the auxiliary subunits. (a) Channel complex is composed of the pore-forming $Ca_V a_1$ and the auxiliary $Ca_V a_2 \delta$, $Ca_V \beta$, and $Ca_V \gamma$ subunits. The $Ca_V a_2 \delta$ and $Ca_V \gamma$ subunits contain transmembrane domains, whereas $Ca_V \beta$s are intracellular. (b) The $Ca_V a_1$ subunits consist of four transmembrane domains (I–IV) and the linker joining I and II encompasses the α-interaction domain (AID). (c)The $Ca_V \beta$ subunits are formed by three conserved domains: PSD95/Dlg1/ZO-1 (PDZ), Scr homology 3 (SH3), and guanylate kinase (GK). The $Ca_V \beta$ subunit interaction domain (BID) is one of the regions involved in the interaction of the protein with the $Ca_V a_1$ subunit. (d) The $Ca_V a_2 \delta$ subunits consist of a_2 (blue), which is an extracellular subunit, disulphide-bonded to the δ subunit (orange), which is membrane-associated. The approximate positions of the vWA domain and the two bacterial chemosensory domains (Cache; C) are also indicated. The sites of interaction between the $Ca_V a_1$ subunit and the $Ca_V a_2 \delta$ subunit are unknown.

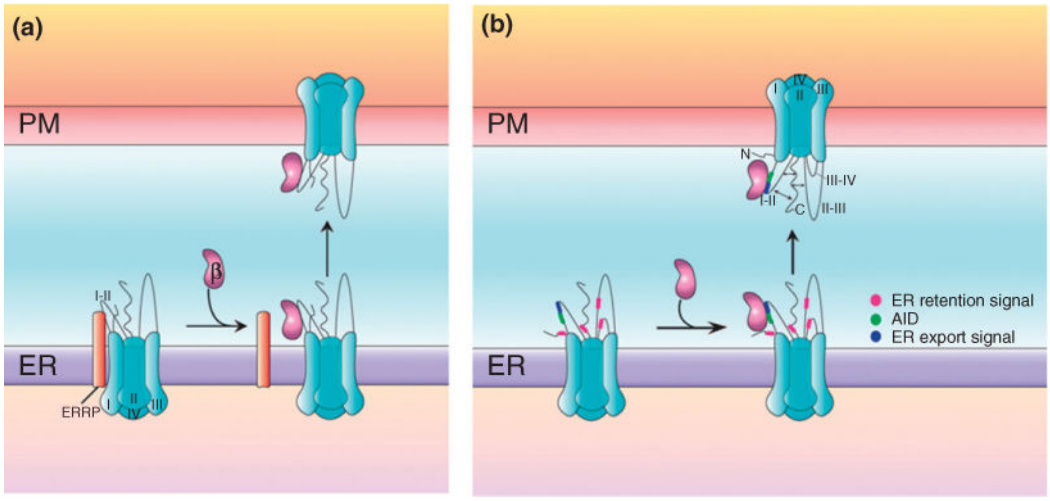

FIGURE 2.

Two distinct models for $Ca_V\beta$ subunit-induced increase in Ca_V channel expression at the plasma membrane. (a) In the absence of $Ca_V\beta$, the $Ca_V\alpha_1$ subunit remains trapped within the endoplasmic reticulum (ER) by binding via the I-II loop onto an ER retention protein (ERRP) of unknown identity. Expression and subsequent binding of the $Ca_V\beta$ subunit to the I-II loop relieves the trafficking clamp imposed by the ERRP and allows Ca_V channel complexes to be targeted to the cell surface. (b) In the absence of $Ca_V\beta$, an ER export signal (blue) present on the $Ca_V\alpha_1$ subunit I-II loop is functionally overcome by ER retention signals (pink) present in different regions of the protein, leading to channels being retained in the ER. Upon $Ca_V\beta$ binding to the $Ca_V\alpha_1$ subunit, a C-terminus-dependent conformational change of the intracellular domains occurs that diminishes the strength of ER retention signals leading to channel transport to the plasma membrane.

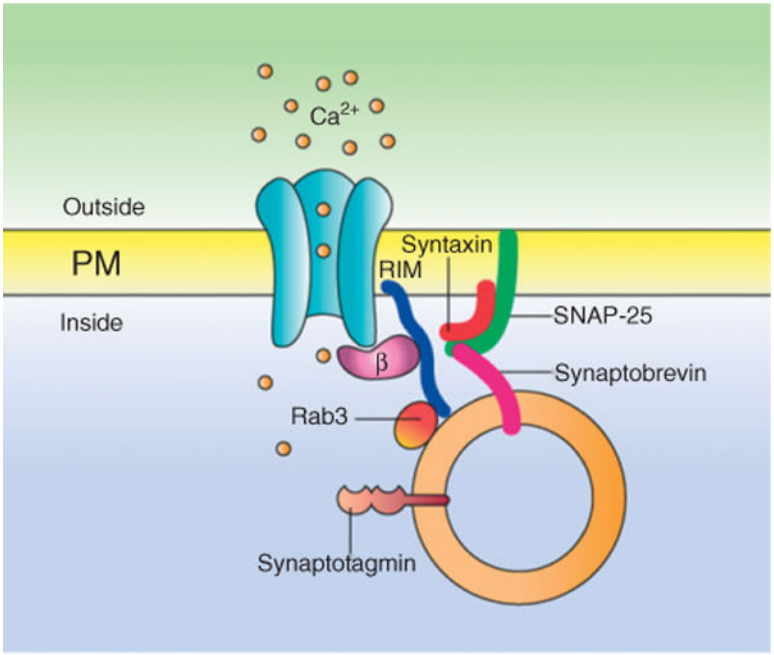

FIGURE 3.

Functional coupling between Ca_V channels and secretory vesicles via the $Ca_V\beta$ subunit. (a) RIMs anchor synaptic vesicles next to channels through its interaction with zone-specific proteins (Rab3) and the $Ca_V\beta$ subunit. After depolarization, RIMs regulate the time course of channel inactivation resulting in a sustained Ca^{2+} influx. This molecular organization favors hormone and neurotransmitter release.

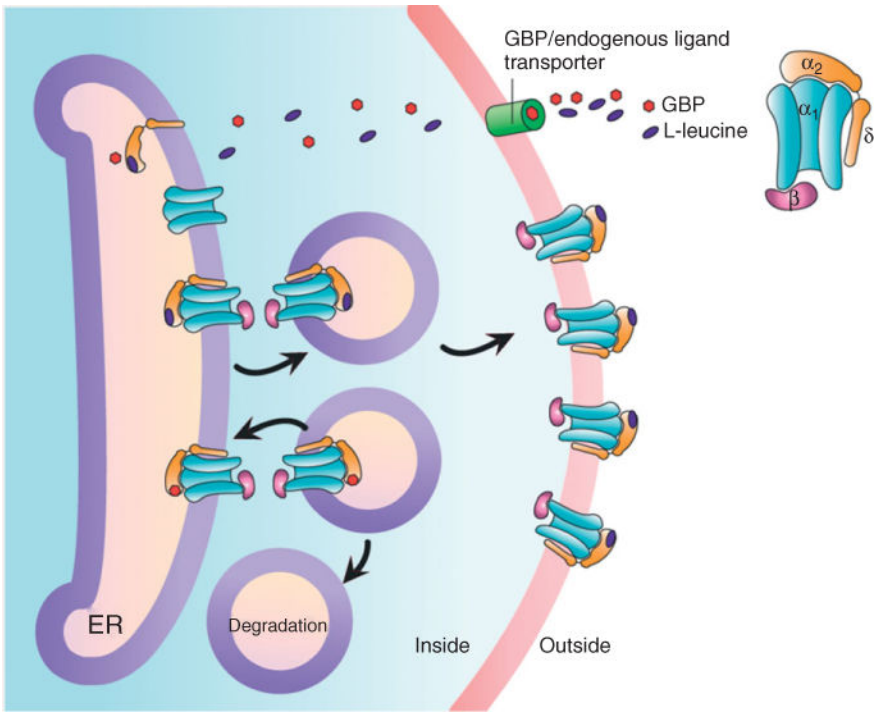

FIGURE 4.

Hypothetical mechanisms of action of gabapentin (GBP). The effect of GBP may be to displace an endogenous ligand (L-leucine) and impair the ability of the auxiliary $Ca_V a_2\delta$ subunit to increase the number of functional channels at the plasma membrane. GBP may be entering the cells using the system-L transporter protein LAT4 and might be exerting its effect on intracellular $Ca_V a_2\delta$ subunits during assembly and trafficking of the Ca_V channel complex to the cell surface.

TABLE 1

$Ca_V a_1$ subunits can be divided into three classes according to amino acid sequence identity, as shown in the dendrogram. The Ca_V1 and Ca_V2 classes are termed High-Voltage-Activated (HVA) channels. The Ca_V3a_1 subunits form the Low-Voltage-Activated (LVA) channels. The original names, molecular nomenclature, and type of currents are given in purple, yellow, and pink, respectively.