Voltage-gated calcium channels and their auxiliary subunits: physiology and pathophysiology and pharmacology

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Abstract  Voltage-gated calcium channels are essential players in many physiological processes in excitable cells. There are three main subdivisions of calcium channel, defined by the pore-forming $\alpha_1$ subunit, the CaV1, CaV2 and CaV3 channels. For all the subtypes of voltage-gated calcium channel, their gating properties are key for the precise control of neurotransmitter release, muscle contraction and cell excitability, among many other processes. For the CaV1 and CaV2 channels, their ability to reach their required destinations in the cell membrane, their activation and the fine tuning of their biophysical properties are all dramatically influenced by the auxiliary subunits.
that associate with them. Furthermore, there are many diseases, both genetic and acquired, involving voltage-gated calcium channels. This review will provide a general introduction and then concentrate particularly on the role of auxiliary \(\alpha_2\delta\) subunits in both physiological and pathological processes involving calcium channels, and as a therapeutic target.

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**Abstract figure legend** Diagram of a presynaptic terminal showing a Ca\(_{\text{v}2}\) calcium channel and associated GPCR.

**Abbreviations** AID, \(\alpha\)-interaction domain; AP-1, adaptor protein complex-1; BBS, bungarotoxin binding site; BTX, \(\alpha\)-bungarotoxin; DRG, dorsal root ganglion; EM, electron microscopy; ER, endoplasmic reticulum; GK, guanylate kinase; GPCR, G-protein coupled receptor; GPI, glycosyl-phosphatidylinositol; HIV, human immunodeficiency virus; MIDs, metal ion-dependent adhesion site; PMCA, plasma membrane Ca\(^{2+}\) ATPase; RyK, ryanodine receptor; SERCA, sarcoplasmic and endoplasmic reticulum Ca\(^{2+}\) ATPase; SH3, src homology-3; SNP, single nucleotide polymorphism; VWA, Von Willebrand Factor-A domain.

**Introduction**

Excitable cells contain functional voltage-gated ion channels, including calcium channels. Neurons and muscle cells are conventionally excitable, but many other cell types show oscillatory changes in voltage, dependent on the interplay between voltage-gated and calcium-dependent channels (for example see Hu et al. 2012). Free intracellular Ca\(^{2+}\) is maintained at 10–100 nM in the cytoplasm, a low level relative to the extracellular milieu. Voltage-gated calcium channels then react to membrane depolarization by opening, and thus allowing Ca\(^{2+}\) entry, particularly but not exclusively through voltage-gated calcium channels, provides an elevation of intracellular calcium ion concentration, to drive many processes. These include hormone secretion, neurotransmitter release, calcium-dependent transcription of a variety of genes, and also spontaneous pacemaker activity in some neurons, muscles and secretory cells (Mangoni et al. 2003; Guzman et al. 2009; Putzier et al. 2009; Hu et al. 2012; Striessnig et al. 2015). The present review concentrates particularly on the roles of the accessory \(\alpha_2\delta\) subunits. For more comprehensive coverage of calcium channel function, the reader is directed to other recent reviews (Striessnig et al. 2014; Zamponi et al. 2015; Zamponi, 2016).

**Voltage-gated calcium channel subunits**

Functional voltage-gated calcium channels are composed of pore-forming \(\alpha_1\) subunit proteins, encoded by the CACNA1x genes (for review see Catterall et al. 2003), of which there are 10 isoforms in the mammalian genome. In the case of the Ca\(_{\text{v}1.1}\)–Ca\(_{\text{v}1.4}\) channels (known as \(L\)-type channels), these are encoded by CACNA1S, -C, -D and -F, respectively, and also known as \(\alpha_1\), \(\alpha_1\), \(\alpha_1\), \(\alpha_1\)D and \(\alpha_1\)F. The Ca\(_{\text{v}2.1}\)–Ca\(_{\text{v}2.3}\) channels (termed P/Q -, N- and R-type from physiological experiments: Nowycky et al. 1985; Mintz et al. 1992; Piedras-Rentería & Tsien, 1998) are encoded by CACNA1A, -B and -E, respectively, and also known as \(\alpha_1\), \(\alpha_1\)B and \(\alpha_1\)E. The T-type Ca\(_{\text{v}3}\) channels (encoded by CACNA1G, -H and -I) are also termed \(\alpha_1\)G, \(\alpha_1\)H and \(\alpha_1\)I (Cribbs et al. 1998; Perez-Reyes et al. 1998). They are much more similar to each other than to the Ca\(_{\text{v}1}\) and Ca\(_{\text{v}2}\) channels (Fig. 1).

Although the \(\alpha_1\) subunits dictate the principal biophysical and pharmacological properties of these channels, their expression is enhanced and their properties are modified by the two main auxiliary (or accessory) subunits (Tanabe et al. 1987; Mikami et al. 1989; Mori et al. 1991; Varadi et al. 1991). The \(\alpha_2\delta\) and \(\beta\) subunits also play important roles in channel folding and their subsequent transport to the cell surface, and into particular domains of polarized cells such as neurons. These processes are together known as trafficking, and involve multiple steps. Both the Ca\(_{\text{v}1}\) and Ca\(_{\text{v}2}\) classes of channels are able to form a heteromeric complex, co-assembling with one of four \(\beta\) subunits (encoded by CACNB1—4; Fig. 2A and B), and one of four \(\alpha_2\delta\) subunits (encoded by CACNA2D1—4; Fig. 2A and C). For the Ca\(_{\text{v}3}\) channels, the \(\alpha_1\) subunits can form functional channels alone, but may also associate with other proteins.

All of the \(\alpha_1\), \(\beta\) and \(\alpha_2\delta\) subunits form a large number of variants as a consequence of alternative splicing events. This opens the potential for a huge diversity of properties and function. A \(\gamma\) subunit also forms part of the skeletal muscle calcium channel complex, which comprises Ca\(_{\text{v}1.1}\), \(\beta_1\)a, \(\gamma_1\) and \(\alpha_2\delta\)-1 (Jay et al. 1990). However, although multiple other \(\gamma\) subunits have been cloned (Letts et al. 1998; Moss et al. 2002; Tomita et al. 2003), no \(\gamma\) subunits have been shown to form an integral part of cardiac (Walsh et al. 2009) or neuronal (Moss et al. 2002; Müller et al. 2010) calcium channels. In contrast, they have well-defined roles as transmembrane AMPA-glutamate receptor modifying proteins (Tomita et al. 2009).
Furthermore, for some CaV1 and CaV2 calcium channels, the tight binding of calmodulin to the so-called ‘IQ’ domain in their C-terminal tail allows calmodulin to be considered as a quasi-subunit (Mori et al. 2008; Kim et al. 2010; Ben-Johny et al. 2013).

Voltage-gated calcium channel localization

CaV1.1 is the only isoform present in mammalian skeletal muscle t-tubules, and shows very low expression elsewhere, including brain (Bannister & Beam, 2013). CaV1.2 is the main isoform in ventricular cardiac muscle, and is also present in smooth muscle cells, secretory tissue and the nervous system (Striessnig et al. 2014). CaV1.3 has a more limited localization than CaV1.2, playing a major role in sinoatrial node tissue, and in the auditory system (Platzer et al. 2000; Mangoni et al. 2003; Baig et al. 2011), although it is also present in brain. It is also present in some endocrine tissues, including aldosterone-secreting cells of the adrenal gland, where somatic mutations give rise to resistant hypertension (Azizan et al. 2013; Scholl et al. 2013). CaV1.4 shows very restricted distribution, particularly in the visual system (Mansergh et al. 2005).

The CaV2.x channels show a primarily neuronal distribution and are involved in fast neurotransmitter release (Takahashi & Momiyama, 1993; Wu et al. 1999; Cao & Tsien, 2010). CaV2.1 channels are present throughout the brain, and are particularly prevalent in cerebellum (Ophoff et al. 1996), where they make up the predominant calcium current in Purkinje neurons (Mintz et al. 1992; Westenbroek et al. 1995). They are involved in neurotransmission in most mature mammalian central synapses (Westenbroek et al. 1995; Iwasaki et al. 2000, 2005; Nakamura et al. 2015). CaV2.2 is distributed throughout the central (Westenbroek et al. 1992) and peripheral nervous systems (Lipscombe et al. 1988; Boland et al. 1994; Wheeler et al. 1994). It is particularly important for neurotransmission early in mammalian development, although it co-exists with CaV2.1 in most mature synapses (Iwasaki et al. 2000). CaV2.2 also plays a dominant role in the mature peripheral nervous system (Chaplan et al. 1994; Bowersox et al. 1996). CaV2.3, although originally described as being low voltage activated (Soong et al. 1993), is thought to correspond to residual R-type calcium current (Zhang et al. 1993; Tottene et al. 2000; Wilson et al. 2000). It is present in many brain regions and is found both pre- and postsynaptically in neurons (Parajuli et al. 2012). CaV2.3 has been found to be involved in spontaneous release of glutamate (Ermolyuk et al. 2013), although the CaV2.3 blocker SNX-482 also blocks some K+ channels, making dissection of its physiological functions more difficult (Kimm & Bean, 2014).

The CaV3 channels are extensively distributed in neurons and other excitable cells (Cribbs et al. 1998; Perez-Reyes, 1998; Perez-Reyes et al. 2009). For example, they are prevalent in the thalamus (Perez-Reyes, 2003), and also have important roles in primary afferent pathways (Francois et al. 2015; Gadotti et al. 2015; for recent review see Zamponi et al. 2015). They have important roles in neuronal and cardiac excitability and in cardiac and neuronal pacemaker activity (Perez-Reyes, 2003; Guzman et al. 2009; Putzier et al. 2009). In some synapses they also have a presynaptic function in transmitter release (Huang et al. 2011; Carbone et al. 2014).

Association of α1 subunits with auxiliary subunits

Biochemical isolation of calcium channels has indicated that native L-, N- and P/Q-type channels in muscle and brain are all associated with β and α2δ subunits (Tanabe et al. 2003). Furthermore, for some CaV1 and CaV2 calcium channels, the tight binding of calmodulin to the so-called ‘IQ’ domain in their C-terminal tail allows calmodulin to be considered as a quasi-subunit (Mori et al. 2008; Kim et al. 2010; Ben-Johny et al. 2013).

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et al. 1987; Witcher et al. 1993; Liu et al. 1996). However, it has been noted that the association of the $\alpha_2\delta$ subunit with the channel complex is more easily dissociated by the detergents used during purification than the interaction of the $\beta$ subunit (Jay et al. 1991; Gee et al. 1996; Müllner et al. 2010). It is also possible that not all native calcium channel complexes contain an $\alpha_2\delta$ subunit. By contrast, the association between the $\alpha_1$ and $\beta$ subunits is quite robust, and shows a high affinity for interaction with the intracellular loop between domains I and II of Cav1 and Cav2 channels (Pragnell et al. 1994; Canti et al. 2001; Van Petegem et al. 2004). Despite this difference, both the $\beta$ and $\alpha_2\delta$ subunits increase the expression and function of these channels, as described below.

**Structural information on voltage-gated calcium channels**

There is detailed structural information concerning the cytoplasmic $\beta$ subunits. Initially, a modelling study showed that $\beta$ subunits contained a core SH3 and guanylate kinase-like (GK) domain (Hanlon et al. 1999; Fig. 2B). This was confirmed in X-ray crystallographic studies of the SH3-GK core domains of three calcium channel $\beta$ subunits, in association with an interacting peptide derived from the I-II linker (Chen et al. 2004; Opatowsky et al. 2004; Van Petegem et al. 2004). From these and other studies, the GK domain is seen to bind to the $\alpha$-interaction domain (AID) which is in the proximal part of the I-II linker (Fig. 2A). The $\beta$ subunit is thought to promote the formation of an $\alpha$-helix, in the AID motif, extending back to the end of S6 in domain I (Opatowsky et al. 2004; for reviews see Richards et al. 2004; Buraei & Yang, 2010). This is likely to promote folding to form mature channels.

More recently, very valuable crystallographic information pertaining to the $\alpha_1$ subunit structure has come from studies of the bacterial single domain sodium channel NaVAb, whose structure was solved by X-ray crystallography (Payandeh et al. 2011). Subsequently, key residues in the pore of this channel were mutated to render the channel Ca$^{2+}$ permeable (Tang...
et al. 2014); this structure was able to provide detailed information about the Ca$^{2+}$ permeation pathway. There are also structures of calmodulin interacting with the proximal C-terminus of Cav1.2 (Kim et al. 2008, 2010), revealing the nature of this interaction and shedding light on the mechanism of Ca$^{2+}$-dependent inactivation.

The initial low resolution single particle electron microscopy (EM) structures of the L-type calcium channel complex, also called the dihydropyridine receptor, from skeletal muscle (Serysheva et al. 2002; Wolf et al. 2003; Wang et al. 2004; Hu et al. 2015) and cardiac muscle (Walsh et al. 2009), showed an asymmetric structure, with a density identified as $\alpha_\delta$ extending out from the complex. More recently a high resolution cryo-EM structure of the Cav1.1 calcium channel complex purified from skeletal muscle has now provided us with much greater detail, at near atomic resolution, particularly regarding the transmembrane organization and pore of the $\alpha_1$ subunit, and the orientation of the $\alpha_\delta$ subunit domains (Wu et al. 2015). It has shown a clockwise arrangement of the $\alpha_1$ subunit domains, and identified that there are multiple interactions of $\alpha_2\delta$-1 subunit with the extracellular loops of domains I-III of the $\alpha_1$S subunit.

Modulation of calcium channel function by second messengers and G proteins

There is insufficient space in this review to cover the enormous amount of information on multiple second messenger effects on calcium channel function. Three key areas that can be highlighted are firstly: Ca$^{2+}$-dependent inactivation and facilitation of Cav1.2, Cav1.2 and Cav1.3 channels, by interaction with calmodulin associated with the C-terminal tail of the $\alpha_1$ subunit (Dick et al. 2008; Minor & Findeisen, 2010; Ben-Johny et al. 2013). Secondly, there is an important phosphorylation process that is responsible for $\beta$-adrenergic stimulation of cardiac calcium currents (Reuter, 1987; Fuller et al. 2010). The mechanism involves enhancement of Cav1.2 currents by cyclic AMP-dependent protein kinase, which results from phosphorylation-induced relief of auto-inhibition by a peptide cleaved from the channel C-terminus (Fuller et al. 2010; Fu et al. 2013). Thirdly, there is a ubiquitous G-protein coupled receptor (GPCR)-mediated inhibition of the Cav2.2 class of channels mediated by G$\beta$Y (Dolphin, 2003; Zamponi & Currie, 2013).

Regarding the interplay between second messenger modulation and auxiliary subunits, initial studies identified that G$\beta$Y bound to a site on the I-II linker of Cav2 channels that overlapped with the Cav$\beta$ subunit (Zamponi et al. 1997), opening the possibility that they compete for this binding site. We then identified that in the absence of a Cav$\beta$ subunit, G$\beta$Y-mediated inhibition is still present, but it is not voltage dependent, meaning that it cannot be removed by preceding depolarization. Therefore, the presence of the Cav$\beta$ subunit is required for G$\beta$Y-mediated G-protein modulation to show voltage-dependent properties (Meir et al. 2000; Zhang et al. 2008), and a simple competition for binding is not responsible for G$\beta$Y-mediated inhibition. Further to this, we identified key residues within the N-terminus of Cav2 channels that are essential for G-protein modulation (Page et al. 1998; Canti et al. 1999; Leroy et al. 2005), and this work was extended by others (Agler et al. 2005).

Interplay between the action of $\beta$ and $\alpha_2\delta$ subunits in calcium channel function

For both Cav1 and Cav2 channels, the Cav$\beta$ subunits are extremely important for expression of functional channels in several heterologous expression systems (Varadi et al. 1991; Pragnell et al. 1994; Jones et al. 1998; Leroy et al. 2005). Interaction of the $\alpha_1$ subunit with a $\beta$ subunit has a number of consequences. By binding via their guanylate kinase domain (Fig. 2B) to the intracellular AID motif on the $\alpha_1$ subunits (Pragnell et al. 1994; Fig. 2A), they increase folding of the channels and protect the channels from endoplasmic reticulum (ER)-associated proteasomal degradation (Altier et al. 2011; Waithe et al. 2011); thus they allow more channels to reach the plasma membrane (Fig. 3A). However, it is difficult to determine whether $\beta$ subunits are absolutely essential for $\alpha_1$ subunits to reach the cell surface. This suffers from the problem that several expression systems, in particular Xenopus oocytes, express native $\beta$ subunits (Canti et al. 2001). The $\alpha_2\delta$ subunits produce an additional increase in current density, described in more detail below (Fig. 3B). However, because a number of expression systems, including Xenopus oocytes, HEK-293 and the tsA-201 cells derived from them, also contain some endogenous $\alpha_2\delta$-1 (Singer-Lahat et al. 1992; Dolphin et al. 1999; Kadurin et al. 2012a), this also complicates assessment of their role. Nevertheless, both $\alpha_2\delta$ and $\beta$ subunits increase the expression at the plasma membrane of Cav1 and Cav2 channels, and where it has been investigated, some evidence suggests that $\alpha_2\delta$ subunits are poorly effective unless the Cav$\beta$ subunits are also expressed (Cassidy et al. 2014; Fig. 4).

Isoforms and topology of $\alpha_2\delta$

All the $\alpha_2\delta$ proteins have a similar structure (for reviews see Felix, 1999; Davies et al. 2007; Dolphin, 2012; Fig. 2C and D). The N-terminus has a signal sequence directing the nascent polypeptide into the lumen of the ER, such that it becomes extracellular, once transported to the plasma membrane (Fig. 2C). Several domains can be identified in the sequence of $\alpha_2\delta$ proteins, including a Von Willebrand Factor-A (VWA) domain (Whittaker & Hynes, 2002; Fig. 2C). These domains, as well as being present in von Willebrand factor itself, are generally involved in
extracellular protein–protein interactions, dependent on divalent cations, particularly by integrins and extracellular matrix proteins. A key motif in VWA domains is the metal ion-dependent adhesion site (MIDAS), which involves coordination of the divalent cation by a ring of up to five polar or charged residues (Whittaker & Hynes, 2002). If the MIDAS site is ‘perfect’, with the full complement of five residues, it is highly likely to be involved in such protein–protein interactions (Whittaker & Hynes, 2002), and this is the case in α2δ-1 and α2δ-2 (Canti et al. 2005), whereas α2δ-3 and α2δ-4 have one missing polar residue in the MIDAS motif. There is also a region in the α2δ subunits containing so-called Cache domains, which have homology to bacterial chemosensory domains (Anantharaman & Aravind, 2000; Dolphin, 2012). The recent EM structure also identified a Cache domain, N-terminal to the VWA domain (Wu et al. 2015). There are other identified genes with predicted similarity to α2δ subunits, such as CACHD1 (Whittaker & Hynes, 2002) (Fig. 2D), whose functions remain to be determined.

The C-termini of all α2δ subunits all have a hydrophobic region first identified to be a transmembrane domain (Ellis et al. 1988; Jay et al. 1991). This led to the α2δ proteins being described as single pass type I (C-terminal cytoplasmic) transmembrane proteins. From prediction programs we found that at least two of the α2δ subunits (α2δ-3 and α2δ-4) are predicted with high likelihood to

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**Figure 3. Examples of effects of auxiliary subunits on CaV2.2 calcium channel currents**

A, CaV2.2 calcium currents: effect of β subunits. Example of peak CaV2.2 current at 0 mV in the absence of β (blue) and presence of β1b (black). B, CaV2.2 calcium currents: effect of α2δ subunits. Example of peak CaV2.2/β1b current at 0 mV in the absence of α2δ (red) and presence of α2δ-3 (black). Scale bars apply to both A and B. Charge carrier 1 mM Ba2+, expression in tsA-201 cells, as in a previous study (Leroy et al. 2005). C, effect of different α2δ subunits on inactivation. Examples of normalized peak current for CaV2.2–β1b (black), CaV2.2–β1b–α2δ-2 (blue) and CaV2.2–β1b–α2δ-1 (red), over a 2.5 s timescale. Expression in Xenopus oocytes, as in a previous study (Canti et al. 2005).

**Figure 4. CaV2.2 cell surface expression: effects of β1b and α2δ-1**

Cell surface expression of bungarotoxin binding site (BBS) tagged CaV2.2 labelled with α-bungarotoxin (BTX) coupled to AF488 dye (green). Top panel: examples of N2a cells transfected with CaV2.2–β1b–α2δ-1 (left), CaV2.2–β1b (middle) and CaV2.2–α2δ-1 (right). Scale bar 20 μm. Bottom panel: mean (± SEM) data for cell surface expression of CaV2.2, for cells expressing CaV2.2–β1b–α2δ-1 (black bar), CaV2.2–β1b (red bar) and CaV2.2–α2δ-1 (green bar) or CaV2.2 alone (blue bar). Data are taken from a recent study (Cassidy et al. 2014).
be glycosyl-phosphatidyl inositol (GPI)-anchored, partly because the C-terminal hydrophobic domain is very short and present at the extreme C-terminus, as well as the presence of a predicted GPI-anchor ω-site (Pierleoni et al. 2008; Davies et al. 2010). We have provided evidence for this post-translational modification for α2δ-1, α2δ-2 and α2δ-3 (Davies et al. 2010; Alvarez-Laviada et al. 2014). The genes for all the α2δ subunits encode a single precursor protein, which is post-translationally proteolytically processed into two polypeptides (Jay et al. 1991). The α2 and δ polypeptides remain disulfide-bonded together. The cysteines residues involved in this disulfide bonding have been determined for α2δ-1 (Calderon-Rivera et al. 2012). We have recently been studying the relevance of proteolytic processing into α2 and δ to the physiological function of α2δ (Kadurin et al. 2012b, and authors’ unpublished data).

**Effect of α2δ subunits on calcium channels reaching the plasma membrane**

In general, the α2δ subunits have been found to increase the expression (either functional expression or amount of protein on the plasma membrane) of several different CaV1 or CaV2 combinations with β subunits (Shistik et al. 1995; Gurnett et al. 1996; Felix et al. 1997; Wakamori et al. 1999; Gao et al. 2000; Yasuda et al. 2004; Canti et al. 2005; Davies et al. 2010). For example, for the CaV2.1–β4 combination, calcium currents were increased 3-fold by α2δ-2. This set of calcium channel subunits is found in cerebellar Purkinje cells, where α2δ-2 is strongly represented (Barclay et al. 2001; Brodbeck et al. 2002). However, α2δ-2 did not alter the single channel conductance, suggesting strongly that the large increase in whole cell current is solely due to an increase in the number of functional channels at the cell surface (Barclay et al. 2001; Brodbeck et al. 2002). However, the term ‘increased number of functional channels’ can indicate increased amount of channel protein in the plasma membrane and/or an increased proportion of the channels already in the plasma membrane able to respond to depolarization. There is strong evidence that the cell surface expression of CaV1 and CaV2 α1 subunits is increased by α2δ subunits (Fig. 4), although the mechanism(s) underlying this increase are still being unravelled (Tran-Van-Minh & Dolphin, 2010; Cassidy et al. 2014).

The situation for CaV2.3 channels is less clear. It has been reported that CaV2.3 produces relatively large currents when expressed alone in Xenopus oocytes (Soong et al. 1993; Schneider et al. 1994; Qin et al. 1998), and α2δ-1 subunits did not increase CaV2.3 currents in this expression system (Qin et al. 1998). Furthermore, in HEK-293 cells α2δ-1 produced a 2-fold elevation of the maximum conductance for CaV2.3 alone, although it gave no additional increase beyond that of β subunits (Jones et al. 1998). Thus it is possible that CaV2.3 may be less affected by α2δ subunits, but this will require confirmation.

Increased trafficking of the calcium channels by these α2δ subunits is highly likely not to be their only mechanism of action. For example, liposomes containing skeletal muscle calcium channel protein exhibited greater calcium flux in the presence of α2δ subunits than in their absence (Gutierrez et al. 1991). Furthermore, the effect of α2δ-1 on CaV2.2 channel density in the plasma membrane when expressed in N2a cells was at the most 2-fold (Cassidy et al. 2014), whereas there was an approximately 10-fold increase in CaV2.2 calcium currents in the presence of α2δ-1 (Hoppa et al. 2012). It has been suggested that α2δ-1 reduced the apparent turnover of CaV2.2, in studies using radiolabelled conotoxin (Bernstein & Jones, 2007), although Cassidy et al. (2014) did not find that α2δ-1 reduced CaV2.2 endocytosis from the plasma membrane in N2a cells.

The perfect MIDAS motif present in the VWA domain of α2δ-1 and α2δ-2 subunits is required for increasing calcium currents (Canti et al. 2005; Hoppa et al. 2012), and also for cell surface expression of CaV2.2 (Cassidy et al. 2014). Mutation of the MIDAS motif also reduced the trafficking of the α2δ subunits themselves, when expressed alone (Canti et al. 2005; Cassidy et al. 2014). This mutation also abolished the capacity of both α2δ-1 (Hoppa et al. 2012) and α2δ-2 (Canti et al. 2005) subunits to increase calcium currents in several expression systems. However, α2δ-3 and α2δ-4 do not contain perfect MIDAS motifs (Whittaker & Hynes, 2002), and may therefore play a smaller trafficking role, despite increasing calcium currents (Davies et al. 2010), by what must be an additional mechanism.

In an early study, it was found that the α2 subunit of α2δ-1 binds to of CaV1.1 domain III (Gurnett et al. 1997). However, the recent structural study shows interaction of α2δ-1 with several extracellular loops in domains I–III of CaV1.1 (Wu et al. 2015). The α2δ-1 MIDAS motif was found to be located immediately above the linker between the first two transmembrane segments in voltage-sensing domain I (Wu et al. 2015). The limited structural evidence for other calcium channels also suggests extensive extracellular contact between CaV1.2 and α2δ-1 (Walsh et al. 2009).
Trafficking of calcium channels to specific membrane domains

The auxiliary α₂δ and β subunits play major roles in the trafficking of CaV1 and CaV2 channels not only to the cell surface, but also to specific domains of polarized cells, including muscle cells and neurons (Dolphin, 2012; D’Arco et al. 2015). We have postulated that the α₂δ subunits are highly likely to interact with proteins involved in trafficking of membrane protein cargoes (Davies et al. 2006; Hendrich et al. 2008; Tran-Van-Minh & Dolphin, 2010). We have found that the α₂δ subunits themselves purify with cholesterol-rich lipid raft domains, and this may influence localization of the calcium channel complexes in plasma membrane microdomains (Davies et al. 2006, 2010; Kadurin et al. 2012a). Interestingly, we have also found that a truncated α₂δ subunit, from which we have removed the C-terminal GPI-anchor motif, is mainly secreted, but nevertheless exhibits some extrinsic plasma membrane association, via interactions that remain to be determined (Kadurin et al. 2012a).

In recent work, we have found that the adaptor protein complex-1 (AP-1) is important for trafficking of CaV2.2 from the trans-Golgi network to the plasma membrane, via an alternatively spliced exon 37 in the proximal C-terminus. The splice variant of CaV2.2 containing exon 37a supports larger currents compared to that containing exon 37b (Castiglioni et al. 2006), and is selectively expressed in nociceptors (Bell et al. 2004). Our work revealed that AP-1 binding motifs, YxxΦ and [DE]xxxL[LI], present only in exon 37a, increase the intracellular trafficking of exon 37a-containing CaV2.2, both to the somatic plasma membrane and into the axons of dorsal root ganglion (DRG) neurons. The ability of exon37a to increase CaV2.2 currents and cell surface density are lost in the absence of α₂δ subunits, suggesting that this auxiliary subunit promotes a particular step in the forward trafficking process (Macabuag & Dolphin, 2015).

Influence of α₂δ subunits on biophysical properties of calcium channels

The α₂δ subunits influence the voltage-dependent and kinetic properties of the calcium currents; in particular they consistently increase the inactivation rate, although to different extents. The effects of α₂δ subunits may also depend on the presence of a particular β subunit.

Activation. In the case of CaV1.2, it was found that α₂δ-1 subunits exerted little effect on the activation voltage dependence (Singer et al. 1991; Welling et al. 1993; Shistik et al. 1995; Bangalore et al. 1996; Shirokov et al. 1998). However, in other studies a hyperpolarization of activation was reported (Felix et al. 1997), and this was also observed from conductance-voltage measurements (Platano et al. 2000). For CaV2.1, co-expressed with β4 in mammalian cells, α₂δ-2 did not affect the voltage dependence of activation (Brodeck et al. 2002). For CaV2.2 co-expressed with β1b, α₂δ-1 increased the activation rate of currents, but had less effect on the voltage dependence of activation (Wakamori et al. 1999). Contrasting results were found for CaV2.3, which shows a greater capacity than CaV1.2 to produce currents in the absence of the auxiliary subunits (Stephens et al. 1997; Qin et al. 1998). For CaV2.3, α₂δ-1 was found to depolarize the activation, in the presence of either β1b or β2a, or in the absence of any β subunits (Qin et al. 1998). In contrast, in another study α₂δ-1 had no effect on the activation voltage dependence for CaV2.3 (Jones et al. 1998).

Inactivation. In some studies, it was found that the α₂δ subunits hyperpolarized the steady-state inactivation for several different calcium channel isoforms (Singer et al. 1991; Felix et al. 1997; Wakamori et al. 1999; Hobom et al. 2000; Canti et al. 2003; Hendrich et al. 2008; Davies et al. 2010), and in α₂δ-1 knockout mice there was a clear depolarization of the steady-state inactivation curve for cardiac calcium channel currents (Fuller-Bicer et al. 2009). However, for CaV2.3 it was found that, whereas β1b caused a hyperpolarization of the steady-state inactivation, α₂δ-1 had no effect on this, either with or without a β subunit (Qin et al. 1998). The α₂δ subunits also increased the rate of inactivation to varying extents, with the greatest effect being observed for α₂δ-1 (Fig. 3C; for review see Canti et al. 2003).

Thus, although the α₂δ subunits affect the kinetics and voltage-dependent properties of the different calcium channel isoforms, there is no clear consensus for the different α₁ and α₂δ isoform combinations. One origin of this complexity may be that there are also usually more mature channels in the plasma membrane in the presence of α₂δ subunits. Such a diversity of effects, although they may appear subtle when measured in isolation, can have important consequences in terms of calcium-and voltage-dependent events in cells, including action potential shape (Hoppa et al. 2012, 2014), and the firing properties of neurons (Margas et al. 2016).

Splice variants of α₂δ subunits

The main α₂δ-1 subunit splice variant present in rat brain is different from that seen in skeletal muscle (Kim et al. 1992). Sequence alignments identified alternative splicing in three regions, called A, B and C (Angelotti & Hofmann, 1996). Our recent study (Lana et al. 2014) indicates that regions A and B are in separate exons, with region A in rat being encoded by exon 18a and region B representing an alternative 3′ splice acceptor site (start site) of exon 19. Region C is also a cassette exon. The main splice variant in rat skeletal muscle is +A +B ΔC, whereas α₂δ-1 (ΔA

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+ B + C) is the principal brain splice variant (Angelotti & Hofmann, 1996; Lana et al. 2014). We have recently shown that it is also the main splice variant in DRG neurons (Lana et al. 2014). However, we also identified a novel minor splice variant (α2δ-1 ΔA + B ΔC) in these neurons (Lana et al. 2014). Alternative splicing of other α2δ subunits has been described in other studies (Barclay & Rees, 2000; Qin et al. 2002).

Distribution of α2δ subunits in the peripheral and central nervous systems

The α2δ-1, α2δ-2 and α2δ-3 subunits are widely expressed in both the peripheral and central nervous system, as documented in a comprehensive in situ hybridization study (Cole et al. 2005). α2δ-1 is present in many neuronal cell types (Cole et al. 2005), including DRG neurons (Newton et al. 2001; Bauer et al. 2009). The α2δ-1 protein is mainly situated in presynaptic terminals, as well as, to smaller extent, in neuronal somata, and also in dendrites (Taylor & Garrido, 2008; Bauer et al. 2009).

The α2δ-1 transcript is expressed preferentially in excitatory compared to inhibitory neurons (Cole et al. 2005). In contrast, α2δ-2 expression was found to be lower than α2δ-1 in most brain regions, with restricted areas showing significant expression, such as the cerebellum (Cole et al. 2005). The distribution of α2δ-2 partially correlates with expression in GABAergic neurons, including cerebellar Purkinje neurons (Barclay et al. 2001; Cole et al. 2005). The α2δ-3 transcript is present throughout the brain, and is particularly prevalent in the caudate-putamen (Cole et al. 2005). It is also present in the auditory system (Pirone et al. 2014) and in the retina (Perez de Sevilla et al. 2015). In contrast, α2δ-4 protein is found in certain endocrine tissues, and is expressed at a low level in the brain (Qin et al. 2002). It also plays a key role in the retina (De Sevilla Muller et al. 2013).

Role of α2δ-1 in neuropathic pain

Neuropathic pain is chronic pain resulting from nerve damage, which may have a number of different underlying causes. Neuropathic pain can be a result of trauma, either directly damaging or impinging on axons. Trigeminal neuralgia, which involves severe facial and jaw pain, is often caused by trapping or pressure on sensory nerves. Cancer-induced neuropathic pain can be also result from direct damage to sensory nerves, or activation of nociceptors as a result of mediators secreted from tumours or in the inflammatory response (Schmidt et al. 2010). Neuropathic pain can also commonly be caused by direct damage to nerves by toxins and drugs. This would include diabetic neuropathy, due to axon damage as a direct result of chronic elevated plasma glucose concentration, and neuropathy caused by cancer chemotherapeutic drugs, for example platinum-based drugs such as cisplatin, microtubule-disrupting taxanes, such as paclitaxel, and vinca alkaloids including vincristine. Some older anti-human immunodeficiency virus (HIV) drugs, such as 2′,3′-dideoxycytidine, can also result in nerve damage and neuropathic pain (Joseph et al. 2004). Viral infection of DRGs can also cause neuralgia, including chronic post-herpetic neuralgia (following shingles), or HIV-induced neuropathic pain, which can be mimicked by injection of the viral coat protein HIV gp-120 (Wallace et al. 2007; Schutz & Robinson-Papp, 2013). Thus both HIV infection and some of the treatments used may initiate neuropathic damage.

Sensory nerve injury results in a change in transcription in those damaged neurons of many genes, which may be either up- or down-regulated, often many-fold (Newton et al. 2001; Wang et al. 2002; Xiao et al. 2002; Dawes et al. 2014). The mechanism of this effect has been investigated for the chemotherapeutic agent paclitaxel and may involve injury-induced modulation of Ca2+ entry and neuronal calcium sensor-1 degradation (Boehmerle et al. 2006, 2007).

Among the large number of genes whose expression is altered, there is a consistent elevation of α2δ-1 mRNA, shown by in situ hybridization (Newton et al. 2001), quantitative PCR (Bauer et al. 2009), microarray analysis (Wang et al. 2002; Xiao et al. 2002) and RNASeq (Perkins et al. 2014). There is an equivalent increase in α2δ-1 protein in DRGs and in the dorsal horn of the spinal cord, shown by immunoblotting (Luo et al. 2001) and immunohistochemistry (Bauer et al. 2009). The increase in α2δ-1 appears to occur in every damaged DRG neuron (Bauer et al. 2009; Patel et al. 2013). In contrast, the levels of CaV2.2 mRNA and protein are not altered in these models (Wang et al. 2002; Li et al. 2006), although a change in splicing of exon 37 has been documented (Altier et al. 2007). This leads to the hypothesis that elevated α2δ-1 results in increased CaV2.2 trafficking to terminals or localization to active zones, thus affecting presynaptic function. Nevertheless, α2δ-1 may also have other roles, for example in neuronal sprouting.

Transgenic mice that overexpress α2δ-1 exhibit a baseline phenotype of allodynia and hyperalgesia (Li et al. 2006), suggesting that the α2δ-1 level in DRG neurons is important for determining the neuropathic response. In agreement with these results, we have shown that in α2δ-1 knockout mice (Fuller-Bicer et al. 2009), there is a marked reduction in baseline responses to mechanical and cold stimulation, and a very retarded hyperalgesic response to sciatic nerve injury, in comparison to wild-type littermate mice (Patel et al. 2013). In agreement with this we found that DRGs from α2δ-1 knockout mice showed strongly reduced ability to fire multiple action potentials (Margas et al. 2016).
We have also recently shown that heterologous over-expression of αδ-1 in cultured DRG neurons (to mimic in vitro the neuropathic state) leads to increased calcium currents and prolonged cytoplasmic Ca\(^{2+}\) responses resulting from membrane depolarization (Fig. 5A). These prolonged Ca\(^{2+}\) transients, once initiated, are not dependent on extracellular Ca\(^{2+}\) but are buffered by mitochondria. Thus, by controlling Cav2.2 channel density in the plasma membrane, possibly at sites where mitochondria and ER are also closely apposed, the αδ-1 subunit has a large effect on depolarization-induced intracellular Ca\(^{2+}\) signalling in DRG neurons (D’Arco et al. 2013; Fig. 5B).

Regarding the involvement of other αδ subunits in the pain pathway, the Drosophila melanogaster CACNA2D3 homologue, straitjacket, was identified as a gene involved in pain processing (Neely et al. 2010).

**Figure 5. Effect of αδ-1 on cytosolic Ca\(^{2+}\) levels**

A, overexpression of αδ-1 in DRG neurons increased the width of depolarization-induced intracellular calcium transients, measured using Fura-2, induced by 100 Hz electrical stimulation (indicated by the bar). Data are taken from a recent study (D’Arco et al. 2015). B, cartoon of localization of Cav2.2 (N-type) calcium channels in the plasma membrane near to ER and mitochondria. Ca\(^{2+}\) is taken up into ER via the sarcomplasmic–endoplasmic reticulum Ca\(^{2+}\)-ATPase (SERCA) pump, and can be released by ryanodine receptor (RyR) activation. Ca\(^{2+}\) is pumped out of cells by the plasma membrane Ca\(^{2+}\)-ATPase (PMCA). Cartoon adapted from a recent study (D’Arco et al. 2015).

**Role of αδ subunits in epilepsies**

Prior to the identification of αδ-1 as the receptor for gabapentin (see below), this drug was known to be of use in the treatment of some forms of epilepsy, as an adjunct drug to improve seizure control (Marson et al. 2000). Gabapentin binds to both αδ-1 and αδ-2, but not to the other αδ subunits. Subsequently, we found, together with Michele Rees and Mark Gardiner, that the mutant mouse strains ducky and ducky\(^{2J}\) involved disruption of the cacna2d2 gene (Barclay et al. 2001). These mice display paroxysmal dyskinesia and absence seizures. Although the mutations are different in the two mouse strains, being a complex rearrangement of the gene in ducky and a two base pair deletion in ducky\(^{2J}\), no full length αδ-2 protein is produced in either strain (Barclay et al. 2001; Brodbeck et al. 2002; Donato et al. 2006). Another mutant mouse, entla, with a similar epileptic phenotype, was then identified and found to have a duplication of exon 3 in cacna2d2 (Brill et al. 2004). Mice with a targeted gene deletion in cacna2d2 also show an epileptic and ataxic phenotype (Ivanov et al. 2004). The mutation in ducky and ducky\(^{2J}\) mice is associated with abnormal morphology of the Purkinje cells (Brodbeck et al. 2002) and markedly attenuated spontaneous activity in these neurons (Donato et al. 2006).

Two human family pedigrees have recently been investigated, in which homozygous recessive mutations in CACNA2D2 resulted in infantile epileptic encephalopathy (Edvardson et al. 2013; Pippucci et al. 2013). The carriers of a single copy of the mutations had no phenotype, in agreement with the absence of phenotype in mice heterozygous for cacna2d2 expression (Barclay et al. 2001).

For αδ-1, no central phenotypes have been identified with any certainty in humans, possibly because most neurons contain more than one subtype of αδ subunit, and these proteins may have a partially interchangeable function. However, CACNA2D1 has been identified as a candidate gene associated with some cases of West syndrome, an early-onset epileptic encephalopathy (Hino-Fukuyo et al. 2015). The CACNA2D1 locus has also been implicated in three patients investigated with intellectual disability and epilepsy, although these patients had deletions that also affected other genes (Vergult et al. 2015).

**Night blindness**

Mutations in the gene CACNA2D4, encoding αδ-4, produce photoreceptor dysfunction, resulting in a form of night blindness (Wycisk et al. 2006b). A spontaneously occurring mouse mutation has also been identified in this gene, with a phenotype of autosomal recessive cone dystrophy, again causing night blindness (Wycisk et al. 2006a,b). This emphasizes the importance of αδ-4 in photoreceptor function.
Neuropsychiatric disorders

As we have reviewed recently (Heyes et al. 2015), rare deleterious mutations in many of the calcium channel genes including CACNA2D1, CACNA2D2 and CACNA2D4 have been linked to both bipolar disorder and schizophrenia (Purcell et al. 2014). Furthermore, CACNA2D2 and CACNA2D4 have also been linked to these psychiatric disorders in Genome-Wide Association Studies (Cross-Disorder Group of the Psychiatric Genomics Consortium, 2013). However, most of the single nucleotide polymorphisms (SNPs) that are associated with these disorders are in introns or intergenic regions, and it remains unclear whether the SNPs have any effects to increase or decrease overall expression, or expression of particular splice variants, or otherwise alter the function of the gene with which they are associated (Heyes et al. 2015). Nevertheless, it has recently been found that expression of CACNA1S, CACNA2D4 and CACNA1F were increased in hippocampal-like neurons derived from induced pluripotent stem cells in patients with bipolar disorder (Mertens et al. 2015). It is interesting that these particular calcium channel genes normally show very low expression in brain, so their physiological role in hippocampus is unclear.

A CACNA2D3 splice site mutation was identified as one of a large number of ‘likely gene-disrupting mutations’ involved in autism spectum disorders (Iossifov et al. 2012). Other rare germline mutations, introducing premature stop codons or aberrant splicing, predicting truncated proteins, have also been found to be associated with autism (Girirajan et al. 2013; De Rubeis et al. 2014). Given the likelihood that autism involves synaptic dysfunction (Malhotra & Sebat, 2012; Ting et al. 2012), it is perhaps not surprising that mutations in α2δ-3, which is present in presynaptic terminals, are found to be one of many potential genetic causes of autism.

Cardiac and endocrine dysfunction

The α2δ-1 protein is strongly expressed together with the L-type calcium channels in skeletal, cardiac and smooth muscle (Ellis et al. 1988; Jay et al. 1991; Klugbauer et al. 1999; Wolf et al. 2003; Walsh et al. 2009). CACNA2D1 mutations have been identified to cause human cardiac dysfunction, including short QT syndrome (Templin et al. 2011) and Brugada syndrome (Burashnikov et al. 2010). The mechanism of disruption resulting from mutations in α2δ-1 has been probed (Bourdin et al. 2015). In agreement with this, disruption of the cacna2d1 gene in mice also caused a cardiac phenotype; the mice exhibited a reduction in basal ventricular myocardial contractility, associated with lower cardiac calcium current density (Fuller-Bicer et al. 2009). Furthermore, mice lacking α2δ-1 also showed reduced pancreatic β-cell calcium currents, and an increased tendency to develop diabetes, particularly on one genetic background (Tuluc et al. 2014).

α2δ subunits as a therapeutic target

The α2δ subunits were discovered to be therapeutic targets completely fortuitously, by virtue of being the unexpected protein target for gabapentin binding. Otherwise they would not have been considered a priori as a relevant drug target, because of the absence of any known ligand or mechanism of action.

Identification of α2δ subunits as gabapentin receptors

Gabapentin and pregabalin were first synthesized as analogues of GABA, with the aim of developing novel anti-epileptic drugs (Taylor et al. 2007; Silverman, 2008). After it was found that they did not act via GABA pathways, purification of the brain 3H-gabapentin ‘receptor’ then resulted in the surprise identification of α2δ-1 (Gee et al. 1996; Brown et al. 1998; Brown & Gee, 1998; Field et al. 2006; Li et al. 2011). 3H-Gabapentin also binds to α2δ-2 (Gong et al. 2001). Several residues in α2δ-1 and α2δ-2 are involved in the binding of the gabapentinoid drugs; one important motif involves three arginine residues, just proximal to the VWA domain (Brown & Gee, 1998; Davies et al. 2006; Field et al. 2006). The binding pocket for gabapentin in α2δ-1 has been further elucidated in the cryo-EM structure (Wu et al. 2015). One may speculate that the basis of the binding of these drugs to α2δ-1 and α2δ-2 subunits might stem from the presence of the Cache domains, and their ancestral role to sense nutrients in bacteria. Furthermore, it is likely that a low molecular weight endogenous ligand might also bind to α2δ-1 and α2δ-2, and be displaced competitively by gabapentin. The binding affinity for 3H-gabapentin increases progressively as the α2δ protein is purified or dialysed, or when isolated in lipid raft fractions, suggesting that an endogenous bound substance that competes with gabapentin binding is being removed (Brown et al. 1998; Davies et al. 2006; Lana et al. 2016). It is also possible that gabapentin binding might disrupt the function(s) of the VWA domain or the Cache domains (Dolphin, 2012; Cassidy et al. 2014). It would be of great interest to determine the nature and function of this endogenous small molecule.

Use of gabapentinoid drugs for epilepsy

Gabapentin is licensed for use as an adjunct drug in several types of epilepsy (Marson et al. 2000) and as a monotherapy in some partial-onset seizures (Glauser et al. 2006). Pregabalin is also effective in the therapy of some epilepsies (for review see Taylor et al. 2007). In order to determine whether α2δ-1 or α2δ-2 was responsible for the anti-epileptic effects of these drugs, experiments
were performed using knock-in mice, engineered to contain a mutant α₂δ-1 or α₂δ-2 with reduced affinity for gabapentinoid drug binding (Field et al. 2006; Lotarski et al. 2011). Pregabalin was not found to be effective against electroshock-induced seizures in mice in which α₂δ-1 subunits are mutated, whereas it was still effective in mice with an equivalent mutation in α₂δ-2 (Lotarski et al. 2014); thus it is likely that the anti-seizure effect of these drugs is primarily via binding to α₂δ-1.

Neuropathic pain and the role of α₂δ subunits

Gabapentin and pregabalin are licensed for use in the treatment of various forms of neuropathic pain (Taylor et al. 2007). In contrast, they have no effect on acute nociceptive pain (Dickenson et al. 2005; Moore et al. 2009). In neuropathic pain models in rodents, it has been shown that binding of the gabapentinoid drugs to α₂δ-1 subunits is required for their therapeutic effect (Field et al. 2006). This finding indicates that binding to α₂δ-2 is not important for the effect of gabapentin, and, indeed, α₂δ-2 was found to be reduced rather than up-regulated in injured DRG neurons (Bauer et al. 2009). Pregabalin is also used in the treatment of fibromyalgia, defined as generalized widespread pain, which may also have a neuro-psychiatric aspect (Smith & Moore, 2012).

In a recent study, we have documented changes in splicing, in addition to overall up-regulation of α₂δ-1, in injured rat DRG neurons (Lana et al. 2014). There was elevated expression of a novel splice variant (α₂δ-1 ΔA + B ΔC), which has a lower affinity for gabapentin (Lana et al. 2014). It is interesting to speculate that variable up-regulation of this, or other, splice variants in people who develop neuropathic pain might be relevant to the inconsistent efficacy of the α₂δ ligand drugs within the patient population.

Calcium channel currents: effects of gabapentinoid drugs

Small acute inhibitory effects of gabapentin have been observed on calcium currents in several systems (Stefani et al. 1998; Martin et al. 2002; Sutton et al. 2002). However, in other studies no acute responses to gabapentin have been reported on native or heterologously expressed calcium channel currents (Schumacher et al. 1998; Davies et al. 2006; Heblich et al. 2008; Hendrich et al. 2008). In DRGs from α₂δ-1-overexpressing mice, it was observed that the calcium currents were rapidly inhibited by gabapentin, whereas this was not the case in wild-type mice (Li et al. 2006). These results imply either that gabapentin is not a direct channel blocker, which would indeed be predicted from the location of its binding site, or that α₂δ-1 is not associated with all the relevant calcium channels in DRGs from wild-type mice.

Calcium channel trafficking: effects of gabapentinoid drugs

We have found that incubation of cultured cells for several hours or days, rather than acute application of gabapentin, produces a reduction of calcium currents, both in expression systems when α₂δ-1 or α₂δ-2 was co-expressed, and also in DRG neurons (Heblich et al. 2008; Hendrich et al. 2008; Tran-Van-Minh & Dolphin, 2010). We observed a corresponding reduction in expression of the α₂δ and associated α₁ subunits on the cell surface (Hendrich et al. 2008; Tran-Van-Minh & Dolphin, 2010;
Cassidy et al. 2014; Fig. 6). We also found that gabapentin reduced forward trafficking of \( \alpha_2\delta-2 \) by inhibiting a post-Golgi trafficking step, in a process requiring Rab11, which is involved in trafficking of cargoes in the recycling endosome compartment (Tran-Van-Minh & Dolphin, 2010). When this pathway was isolated, a response to gabapentin could be observed on a time-scale of 30 min. Furthermore, we observed that chronic administration to nerve-injured rats of an anti-hyperalgesic dosing regimen of pregabalin reduced the elevation in the dorsal horn of presynaptic \( \alpha_2\delta-1 \). We interpreted this effect as being due to reduced axonal trafficking in vivo (Bauer et al. 2009). It is possible that gabapentinoid drugs selectively target calcium channel populations that are rapidly turning over, thus sparing skeletal muscle and cardiac channels, but this will need further experimentation.

**Binding of \( \alpha_2\delta \) subunits to other proteins: effects of gabapentinoid drugs**

In various tissues it has been found that a proportion of \( \alpha_2\delta \) subunits can be purified by biochemical means separately from \( \alpha_1 \) subunits (Gee et al. 1996; Müller et al. 2010), indicating that they may be only loosely associated with \( \alpha_1 \) subunits, or may exist separately. This suggests that they may have other functions in addition to being calcium channel subunits. For example, the \( \alpha_2\delta-3 \) proteins have a documented role in formation of synaptic boutons in Drosophila, which was found to be independent of their involvement with calcium channels, in that it was not mimicked by deletion of the relevant \( \alpha_1 \) subunit (Kurshan et al. 2009). However, since \( \alpha_2\delta \) subunits play a role in trafficking calcium channels, as well as in calcium channel function, it may be that \( \alpha_2\delta \) subunits directly influence the calcium transients which are involved in neurite outgrowth and synapse formation during development (Gu et al. 1994).

Furthermore, the \( \alpha_2\delta-1 \) protein has been found to co-immunoprecipitate with thrombospondins, which are large multi-domain extracellular matrix proteins (Eroglu et al. 2009); although it should be noted that thrombospondins also bind to many other proteins (Kazerounian et al. 2008). In the brain, specific thrombospondins are produced by astrocytes and promote the formation of silent excitatory synapses, lacking postsynaptic receptors (Christopherson et al. 2005). Thrombospondin-induced synaptogenesis was found to require the postsynaptic presence of \( \alpha_2\delta-1 \) (Eroglu et al. 2009). Gabapentin was found to disrupt the in vitro interaction between \( \alpha_2\delta-1 \) and the synaptogenic domain of thrombospondin-2, and also disrupted synaptogenesis, although it had no effect on pre-formed synapses (Eroglu et al. 2009). This effect on synaptogenesis may not be relevant to the main mechanism of action of gabapentin either in neuropathic pain or as an antiepileptic drug, as much synaptic sprouting and remodelling would have taken place before the onset of therapy, although gabapentin could have a protective effect via this mechanism. Nevertheless, it should be emphasized that birth defects were found to be extremely uncommon in babies following chronic gabapentin exposure in the uterus of mothers who were taking the drug as an anti-epileptic medication (Morrow et al. 2006; Molgaard-Nielsen & Hvid, 2011), suggesting that it does not have any significant effect on synapse formation during development in utero.

As a corollary of a potential interaction between \( \alpha_2\delta-1 \) and thrombospondins, we have recently examined whether interaction of thrombospondins with \( \alpha_2\delta-1 \) might influence \( ^3 \)H-gabapentin binding (Lana et al. 2016). We used thrombospondin-4 as it is upregulated in neuropathic pain models (Pan et al. 2015). We found that in membranes from co-transfected cells, thrombospondin-4, significantly reduced the affinity for \( ^3 \)H-gabapentin binding to \( \alpha_2\delta-1 \), in a divalent cation-dependent manner. However, the effect on \( ^3 \)H-gabapentin binding was not reproduced by the synaptogenic domain of thrombospondin-4. Furthermore, we found only weak co-immunoprecipitation of the two proteins, which could not be reproduced with the synaptogenic domain of thrombospondin-4 (Lana et al. 2016). We also could not demonstrate any association between \( \alpha_2\delta-1 \) and thrombospondin-4 on the cell surface of transfected cells, suggesting that the interaction between these two proteins to disrupt \( ^3 \)H-gabapentin binding is occurring in an intracellular compartment of the transfected cells (Lana et al. 2016). It is nevertheless possible that such an interaction might reduce the efficacy of gabapentin in patients.

**Conclusions and future directions**

The \( \alpha_2\delta \) subunits are important auxiliary subunits of the \( \text{Ca}_{\text{v}}1.1 \) and \( \text{Ca}_{\text{v}}1.2 \) voltage-gated calcium channels. They play major roles in trafficking of these channels, both to the plasma membrane and to specific domains, as well as influencing the activation and biophysical properties of these channels. The mechanism of these effects, at a cell biological level, still remains to be determined in detail. They also play a role in the pathology of a number of genetic and other diseases, and represent an important therapeutic target site for drugs. Future therapeutic directions are likely to include identifying selective antagonists distinguishing \( \text{Ca}_{\text{v}}1.3 \) from \( \text{Ca}_{\text{v}}1.2 \) and other L-type channels, finding selective antagonists for the different T-type channels, and understanding better the mechanism of action of the \( \alpha_2\delta \) ligands.

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Additional information

Competing interests

None declared.

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