Regulation of Calcium Channels and Synaptic Function by Auxiliary $\alpha_2\delta$ Subunits

Annette C. Dolphin and Gerald J. Obermair

Abstract

Voltage-gated calcium channels of the Ca_V1 (L-type) and Ca_V2 (N-type, P/Q-type, R-type) classes associate with auxiliary β and $\alpha_2\delta$ subunits that are both important for the function of these channels. While the β subunits are cytoplasmic, $\alpha_2\delta$ subunits are entirely extracellular and provide a critical link between channel and extracellular signaling functions. Here we describe what is known about the structure of $\alpha_2\delta$ subunits, and their importance both for channel trafficking and for their physiological function. We also describe distinct roles of $\alpha_2\delta$ proteins in synapse development and synaptic transmission, potentially beyond their classical role as auxiliary calcium channel subunits. Dysregulation and mutations of specific $\alpha_2\delta$ subunits are associated with several diseases, and $\alpha_2\delta$-1 represents an important therapeutic target for the anti-epileptic and anti-allodynic gabapentinoid drugs. A detailed understanding of specific and potentially redundant $\alpha_2\delta$ functions may pave the way for developing novel treatment options, particularly for neuropsychiatric disorders.

Keywords

Calcium channel auxiliary subunit · Calcium currents · Trafficking · Gabapentin · Excitable cells · Synaptogenesis · Synaptic function · Brain disorders · Drug target · Pain

Abbreviations

AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
Ca_V	voltage-dependent calcium
DRG	dorsal root ganglion
eGFP	enhanced green fluorescent protein
ER	endoplasmic reticulum
GABA	Gamma-aminobutyric acid
GPI	glycosylphosphatidylinositol

A. C. Dolphin (✉)
Department of Neuroscience Physiology and Pharmacology, University College London, London, UK
e-mail: a.dolphin@ucl.ac.uk

G. J. Obermair (✉)
Division Physiology, Department Pharmacology, Physiology and Microbiology, Karl Landsteiner University of Health Sciences, Krems, Austria
e-mail: Gerald.Obermair@kl.ac.at

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gSTED microscopy	gated stimulated emission depletion microscopy
LRP1	low-density lipoprotein receptor-related protein 1
mCherry	monomeric Cherry fluorescent protein
MIDAS	metal ion-dependent adhesion site
NMDA	N-methyl-D-aspartate
PSD95	postsynaptic density protein 95
TSP	thrombospondin
VWA	von Willebrand factor-A

Introduction

Voltage-gated calcium (Ca_V) channels mediate and regulate a variety of functions ranging from muscle contraction, hormone secretion, and synaptic transmission to gene regulation. To provide precise timing and control of the calcium ions entering through Ca_V channels, they operate, with some exceptions, in heteromultimeric complexes with auxiliary and cytoplasmic β and extracellular $\alpha_2\delta$ subunits. At least in skeletal muscle, a transmembrane γ subunit is also part of the channel structure (Wu et al., 2016). The traditional concept of Ca_V channel function envisions the pore-forming subunit as the major determinant of the basic biophysical, pharmacological, and physiological properties (see chapters "Subunit Architecture and Atomic Structure of Voltage-Gated Ca2+ Channels" by William A. Catterall, "Voltage-Gated Calcium Channel Auxiliary β Subunits" by Sergej Borowik and Henry M. Colecraft, "Pharmacology of Voltage-Gated Calcium Channels at Atomic Resolution" by William A. Catterall and "Pharmacology and Structure-Function of Venom Peptide Inhibitors of N-Type (Cav2.2) Calcium Channels" by Md. Mahadhi Hasan et al.). Auxiliary subunits are thought to fine-tune Ca_V channel functions by regulating or modulating the membrane expression, localization, trafficking, and biophysical properties of the channel complex (reviewed in Arikkath & Campbell, 2003; Buraei & Yang, 2010; Dolphin, 2009, 2016; Obermair et al., 2008).

Among the auxiliary subunits, the $\alpha_2\delta$ subunit is unique in that the entire protein is extracellular and highly glycosylated, and hence, it interacts with the extracellularly exposed surface of the α_1 subunit. Importantly, this distinctive position also theoretically enables $\alpha_2\delta$ subunits to link the Ca_V complex with other potential extracellular signaling or scaffolding proteins. Indeed, research over recent years has identified a number of extracellular interaction partners as well as physiological functions, some of which may be independent of the classical interaction with the Ca_V complex. A variety of disorders linked to defective $\alpha_2\delta$ subunit functions provide another line of evidence of key roles for these versatile proteins, and the widely prescribed anti-epileptic and anti-allodynic drugs gabapentin and pregabalin act by binding to specific $\alpha_2\delta$ isoforms. In this chapter, we provide a concise overview of the present state of knowledge about $\alpha_2\delta$ subunits, including $\alpha_2\delta$ structure and tissue distribution, the role of specific structural domains for protein function, and Ca_V channel-dependent and -independent functions. We particularly summarize the mechanisms of action of $\alpha_2\delta$ subunits and discuss recent developments concerning their pharmacology and disease association. Investigating and understanding the detailed pathophysiological mechanisms involving Ca_V complexes and $\alpha_2\delta$ subunits may open the path to the identification of new and specific treatment paradigms.

Discovery of $\alpha_2\delta$ Subunits

The Ca_V channel complex was purified, and its properties first investigated from skeletal and then cardiac muscle, by virtue of the ability of the channel to bind ^{3}H-dihydropyridines (DHPs), which were known to block L-type calcium channels (Cooper et al., 1987; Curtis & Catterall, 1984; De Jongh et al., 1989; Leung et al., 1987; Takahashi et al., 1987; Tanabe et al., 1987). The subunit to which the DHP calcium channel blocker bound was identified to be the channel itself, and termed the α_1 subunit (α_1S, with the "S" referring to **s**keletal muscle). This co-purified with a similar molecular weight but glycosylated

protein, later named $\alpha_2\delta$ (Sharp et al., 1987), and smaller molecular weight β and γ subunits (De Jongh et al., 1989, 1990; Takahashi et al., 1987; Tanabe et al., 1987).

The $\alpha_2\delta$ subunit was so called as it was found to consist of the two proteins α_2 and δ that remain associated, except in reducing conditions when they are clearly separated (De Jongh et al., 1990). These two disulfide-bonded proteins were both highly glycosylated, suggesting they were largely extracellular proteins, and they were also membrane associated, and initially identified as transmembrane proteins, with δ containing a hydrophobic, potentially transmembrane, α-helix (Brickley et al., 1995; Gurnett et al., 1996).

Identification of $\alpha_2\delta$ Subunits

Cloning

The $\alpha_2\delta$-1 subunit was the first $\alpha_2\delta$ whose gene was identified and cloned (C*acna2d1*). This was achieved by first obtaining some peptide sequences from the α_2 and δ proteins purified from skeletal muscle. Once obtained, the $\alpha_2\delta$ sequence then showed that both α_2 and δ subunits were encoded by the same gene, which is therefore translated as a pre-protein, and then enzymatically cleaved into α_2 and δ (De Jongh et al., 1990; Ellis et al., 1988). Two additional genes encoding $\alpha_2\delta$-2 and $\alpha_2\delta$-3 were identified by homology to $\alpha_2\delta$-1 (Ellis et al., 1988), and $\alpha_2\delta$-3 was then cloned (Klugbauer et al., 1999). $\alpha_2\delta$-2 was also cloned by virtue of the fact that it is mutated in a spontaneously arising mouse model of absence epilepsy and ataxia called *Ducky* (Barclay et al., 2001; Barclay & Rees, 2000), and $\alpha_2\delta$-4 was cloned by homology to other $\alpha_2\delta$ sequences (Qin et al., 2002).

Splice Variants

The *Cacna2d1* gene for $\alpha_2\delta$-1 was originally identified to contain three alternatively spliced regions (A, B, and C) within the α_2 moiety (Angelotti & Hofmann, 1996). The rat $\alpha_2\delta$-1 genomic sequence contains 39 exons, and further analysis of this sequence found that A is encoded by exon 18a, B is formed as a result of utilizing an alternative 3′ splice acceptor site for exon 19, while C represents the inclusion of exon 23 (Lana et al., 2014) (Fig. 1). In skeletal muscle, $\alpha_2\delta$-1 (+A + B ΔC) was the only splice variant detected, whereas in heart, multiple different splice variants were found (Angelotti & Hofmann, 1996; Lana et al., 2014). By contrast, in neuronal tissue, including dorsal root ganglion (DRG) neurons, the predominant splice variant is ΔA + B + C (Angelotti & Hofmann, 1996; Lana et al., 2014) (Fig. 1). The mouse *Cacna2d2* gene also contains 39 exons (Barclay & Rees, 2000), and splice region C has been found to be of functional relevance, as it regulates the trans-synaptic recruitment of postsynaptic $GABA_A$ receptors and axonal wiring (Geisler et al., 2019).

Tissue Distribution of $\alpha_2\delta$ Subunits

$\alpha_2\delta$-1

The distribution of $\alpha_2\delta$-1 is widespread, being the main $\alpha_2\delta$ in skeletal, smooth, and cardiac muscle, and it is also present in brain (Angelotti & Hofmann, 1996). It is strongly expressed in DRG neurons (Newton et al., 2001) and is the major $\alpha_2\delta$ isoform in cortical brain regions (Schlick et al., 2010).

$\alpha_2\delta$-2

This species was originally found in multiple tissues, including skeletal and cardiac muscle, pancreas, and brain (Klugbauer et al., 1999, 2003). Within the brain, it is found to be strongly expressed in cerebellum, particularly in Purkinje neurons (Barclay et al., 2001) and, at a lower expression level, also in the cortex (Schlick et al., 2010).

$\alpha_2\delta$-3

This isoform is found mainly in brain (Klugbauer et al., 2003), such as the cortex and hippocampus, and it is the major isoform expressed in the striatum (Geisler et al., 2019).

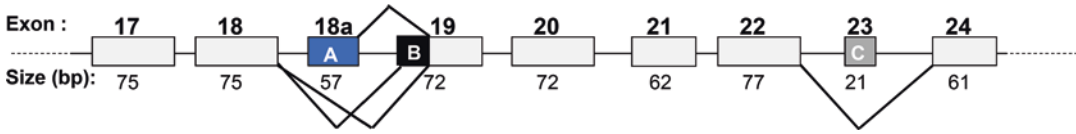

Fig. 1 Splice variants in $\alpha_2\delta$-1. Three alternatively spliced regions (**A**), (**B**), and (**C**) were originally described by Angelotti and Hofmann (1996), and the splice variants were further defined by Lana et al. (2014)

$\alpha_2\delta$-4

The $\alpha_2\delta$-4 isoform is strongly expressed in the retina, particularly at photoreceptor ribbon synapses (Knoflach et al., 2013; Lee et al., 2015; Wycisk et al., 2006a); its expression in the brain, however, seems negligible (Schlick et al., 2010).

Structure of $\alpha_2\delta$ Subunits

Biochemical and Bioinformatic Studies

Following cloning, the primary $\alpha_2\delta$ sequence showed that the N-terminus of $\alpha_2\delta$-1 had a predicted signal sequence; therefore signifying it was extracellular (Fig. 2a). The C-terminus of $\alpha_2\delta$-1 had a hydrophobic domain, suggesting it is transmembrane, with a very short potentially intracellular sequence. Furthermore, the existence of multiple (16–18) glycosylation sites (Fig. 2a, b), and antibody mapping indicated the protein is mainly extracellular (Brickley et al., 1995; Gurnett et al., 1996). Experimentally, both reducing conditions and deglycosylation are important tools to distinguish between $\alpha_2\delta$ and α_2 in immunoblots, particularly in expression studies (Fig. 2b), and the low molecular weight δ can also be identified separately (Davies et al., 2010; De Jongh et al., 1990; Jay et al., 1991). Although almost all $\alpha_2\delta$ in native tissues is proteolytically cleaved, uncleaved $\alpha_2\delta$-1 was identified in DRG neuron cell bodies (Kadurin et al., 2016). Biochemical and other studies also showed disulfide bonding between α_2 and δ (Calderon-Rivera et al., 2012; De Jongh et al., 1990).

GPI Anchoring

It was found that $\alpha_2\delta$ subunits were strongly expressed in lipid rafts (Davies et al., 2006), and subsequently, it was identified that the $\alpha_2\delta$ subunits were not transmembrane, but rather glycosylphosphatidylinositol (GPI) anchored, using both bioinformatic and biochemical techniques (Davies et al., 2010). This GPI anchoring has been confirmed from the structure of $\alpha_2\delta$-1 in the $Ca_V1.1$ and $Ca_V2.2$ channel complexes (Gao et al., 2021; Wu et al., 2016). GPI anchoring occurs immediately following translation in the endoplasmic reticulum (ER), when the preformed GPI anchor replaces the C-terminal hydrophobic sequence (Hooper, 2001) (Fig. 2a). GPI-anchored proteins have different properties from transmembrane proteins, for example, they are highly mobile and concentrated in cholesterol-rich membrane fractions (termed lipid rafts) (Hooper, 2001; Kadurin et al., 2012), and are rapidly endocytosed and recycled to the plasma membrane (Mayor & Riezman, 2004; Tran-Van-Minh & Dolphin, 2010).

VWA Domain

The presence of a von Willebrand factor-A (VWA) domain in the $\alpha_2\delta$ subunits was identified bioinformatically (Whittaker & Hynes, 2002). The VWA domains were then found to be essential for the function of $\alpha_2\delta$ to enhance Ca_V1 and Ca_V2 calcium currents (Canti et al., 2005) and for synaptic calcium channel localization (Schöpf et al., 2021). The VWA domain was predicted to be involved in interacting with the α_1 subunit, via its metal ion-dependent adhesion site (MIDAS)

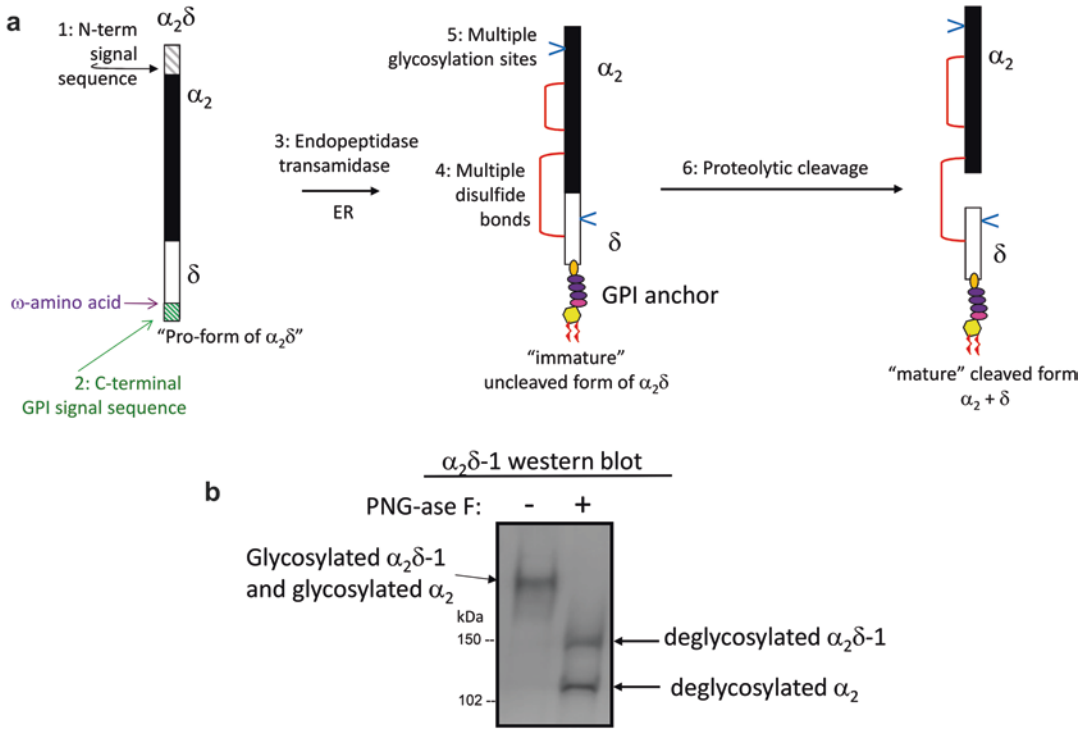

Fig. 2 Post-translational processing of $\alpha_2\delta$ subunits. (**a**) Diagram showing $\alpha_2\delta$ pre-protein (left) and subsequent post-translational processing steps (center), including removal of N-terminal signal sequence during translation (1), GPI anchor attachment within the ER (2, 3), formation of multiple disulfide bonds (4), glycosylation (5), and proteolytic cleavage of $\alpha_2\delta$ into α_2 and δ (6, right). (**b**) Western blot of material from tsA-201 cells transfected with $\alpha_2\delta$-1, showing that $\alpha_2\delta$-1 and cleaved α_2 cannot be clearly differentiated (left lane) unless deglycosylated with PNGase F (right lane). Primary antibody $\alpha_2\delta$-1 monoclonal recognizing an epitope within α_2. (Data courtesy of Dr. Ivan Kadurin)

motif (Canti et al., 2005). This was shown to be the case from the structure of the $Ca_V1.1$ and $Ca_V2.2$ channel complexes, where the MIDAS motif in $\alpha_2\delta$-1 interacts with an aspartate in the first extracellular loop of domain I of the α_1 subunit (Gao et al., 2021; Wu et al., 2016) (Fig. 3a–c). In confirmation of this, mutation of this interaction site in $Ca_V1.2$ and $Ca_V2.2$ also prevented the effect of $\alpha_2\delta$-1 (Bourdin et al., 2017; Dahimene et al., 2018).

Cache Domains

Two Cache domains were identified bioinformatically in $\alpha_2\delta$ subunits (Anantharaman & Aravind, 2000); these Cache domains are very similar in structure to those identified in bacterial chemoreceptors and chemotransducers, where they are often involved in nutrient sensing and chemotaxis (Gumerov et al., 2022). The cryo-EM structure has identified four Cache domains in $\alpha_2\delta$-1 (Fig. 3a, b).

Proteolytic Maturation of $\alpha_2\delta$

The $\alpha_2\delta$ pre-protein encodes α_2 at its N-terminus, followed by the shorter δ sequence. Following translation in the ER, the uncleaved $\alpha_2\delta$ begins to be cleaved in the Golgi apparatus (Kadurin et al., 2017), but the two "subunits" remain associated by pre-formed disulfide bonds, created as the protein folds in the ER. Proteolytic cleavage of $\alpha_2\delta$ appears to represent an activation step for

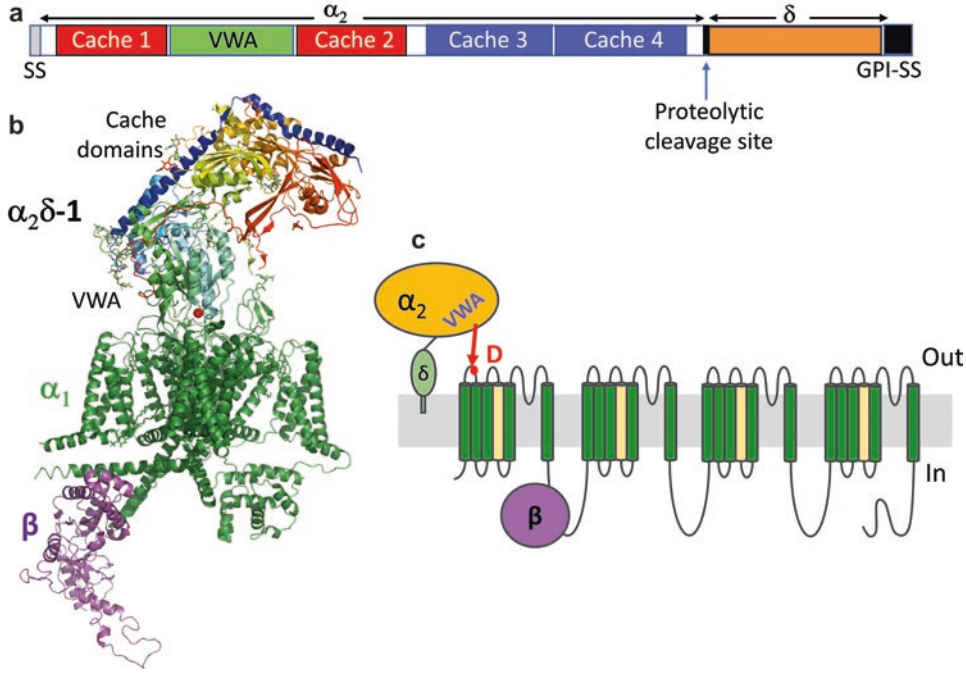

Fig. 3 Domains in $\alpha_2\delta$-1 and interaction with the α_1 subunit. (**a**) Domain structure for $\alpha_2\delta$ showing four Cache domains, with the VWA domain inserted between Cache 1 and Cache 2. (**b**) Cryo-EM structure of the $Ca_V2.2$ complex (Gao et al., 2021), showing the α_1 subunit (green), the β_3 subunit (magenta), and the $\alpha_2\delta$-1 subunit (rainbow). The MIDAS interaction site of the $\alpha_2\delta$-1 VWA domain with the α_1 subunit contains a co-ordinating divalent cation (red sphere). Image prepared from pdb 7miy using Pymol. (**c**) Diagram showing interaction site of the MIDAS motif in the VWA domain of α_2 with an aspartate residue (D) in the first extracellular loop of the α_1 subunit

Ca_V channel function (Ferron et al., 2018; Kadurin et al., 2016) (Fig. 2a).

Cachd1 Protein

Cachd1 was initially identified bioinformatically (although misnamed) (Whittaker & Hynes, 2002) and later confirmed as an $\alpha_2\delta$-like protein (Cottrell et al., 2018; Dahimene et al., 2018) containing an imperfect VWA domain and Cache domains. However, the MIDAS motif in the VWA domain of Cachd1 is highly disrupted and non-functional in terms of enhancement of $Ca_V2.2$ function (Dahimene et al., 2018). Cachd1 also produced differential functional effects on particular calcium channels (see section "Cachd1 function") (Cottrell et al., 2018; Dahimene et al., 2018).

Molecular Structure of $\alpha_2\delta$-1

The predicted domains and post-translational modifications in $\alpha_2\delta$ proteins are all supported by the structure of $\alpha_2\delta$-1 in the skeletal muscle calcium channel ($Ca_V1.1$) complex, which was obtained by cryo-electron microscopy (EM) (Wu et al., 2016). In summary of the salient points, the VWA domain was found to interact with an aspartate on the first extracellular loop on the α_1S calcium channel, which coordinated divalent cation binding with the MIDAS motif in $\alpha_2\delta$-1. No transmembrane domain was detected for $\alpha_2\delta$, in agreement with the evidence that it is GPI anchored (Davies et al., 2010). Four Cache domains were identified in $\alpha_2\delta$, with the VWA domain inserted between the first two (Wu et al., 2016). The structure of $\alpha_2\delta$-1 was found to be

very similar in the $Ca_V2.2$ complex obtained following expression of the subunits in HEK293T cells, and subsequent purification of the complex (Gao et al., 2021).

Functions of $\alpha_2\delta$ Subunits as Calcium Channel Subunits

Effects of Cloned $\alpha_2\delta$ Subunits on Calcium Currents

In most studies $\alpha_2\delta$ subunits have been found to increase Ca_V1- and Ca_V2-mediated currents, although this function is partly dependent on the α_1 isoform, as knockdown of $\alpha_2\delta$-1 in skeletal muscle cells did not reduce $Ca_V1.1$-mediated currents (Meyer et al., 2019; Obermair et al., 2005), whereas the effect on Ca_V2 channel current density was more pronounced (Barclay et al., 2001; Canti et al., 2005; Hendrich et al., 2008; Obermair et al., 2008). Co-expression studies showed that $\alpha_2\delta$-1 increased Ca_V calcium currents that were mediated, for example, by $Ca_V2.1/\beta4$ (Gurnett et al., 1996, 1997), $Ca_V1.2/\beta2a$, and $Ca_V2.3/\beta3$ (Klugbauer et al., 1999; Yamaguchi et al., 2000). Moreover, for $Ca_V1.2$, $Ca_V2.2$, and $Ca_V2.3$, the different auxiliary β subunits were found to regulate Ca_V current properties synergistically with $\alpha_2\delta$-1 in a β subunit-specific manner (Yasuda et al., 2004). Similarly, $\alpha_2\delta$-2 increased $Ca_V2.1/\beta4$ calcium currents (Barclay et al., 2001; Brodbeck et al., 2002) as well as $Ca_V1.2/\beta1b$ and $Ca_V2.2/\beta1b$ currents (Canti et al., 2005). The $\alpha_2\delta$-3 subunit also increased $Ca_V1.2/\beta2a$ and $Ca_V2.3/\beta3$ (Klugbauer et al., 1999) and $\alpha_2\delta$-4, the least well-studied $\alpha_2\delta$ subunit, increased $Ca_V1.2/\beta3$-mediated calcium influx (Qin et al., 2002). The results of these studies always need to be prefaced by the finding that some heterologous expression systems may contain endogenous calcium currents (Berjukow et al., 1996), as well as auxiliary β (Canti et al., 2001) and $\alpha_2\delta$ subunits (Kadurin et al., 2012).

Effects of $\alpha_2\delta$ Subunits on Biophysical Properties of Calcium Currents

No change in single-channel conductance has been observed that can be attributed to $\alpha_2\delta$ subunits (Brodbeck et al., 2002; Wakamori et al., 1999), indicating the main effects are not on permeation. However, $\alpha_2\delta$-1 was found to reduce the percentage of null sweeps in single-channel recordings of $Ca_V2.2$ (Wakamori et al., 1999) and shRNA knockdown of $\alpha_2\delta$-1 slightly reduced the open probability of $Ca_V1.2$ (Tuluc et al., 2007). The $\alpha_2\delta$ subunits also affect the kinetics of activation and inactivation (Canti et al., 2003; Obermair et al., 2005, 2008; Tuluc et al., 2007; Wakamori et al., 1999), and voltage sensor movement for $Ca_V1.2$ (Savalli et al., 2016) and result in a hyperpolarization of both current activation (Felix et al., 1997; Savalli et al., 2016) and inactivation (Canti et al., 2003).

Effects of $\alpha_2\delta$ Subunits on Calcium Channel Trafficking

$\alpha_2\delta$ subunits have been found to increase the plasma membrane expression of $Ca_V2.2$ (Cassidy et al., 2014). This is the case for both exon 37a and 37b-containing C-terminal splice variants of $Ca_V2.2$ (Macabuag & Dolphin, 2015), which show differential gating (Castiglioni et al., 2006), cell surface expression (Macabuag & Dolphin, 2015), and expression in the pain pathway (Bell et al., 2004). Furthermore, presynaptic clustering of $Ca_V2.1$ and $Ca_V2.2$ was strongly reduced in $\alpha_2\delta$ triple knockout/knockdown neurons (Schöpf et al., 2021).

However, for $Ca_V2.2$, it has been shown that the increase in cell surface expression is generally not to the same extent as the increase in calcium current density, which may be up to 12-fold (Hoppa et al., 2012). The increase in cell surface expression has also been found to be greater for $\alpha_2\delta$-1/2 than for $\alpha_2\delta$-3, and is likely to stem from an increase in net forward trafficking of $Ca_V2.2$,

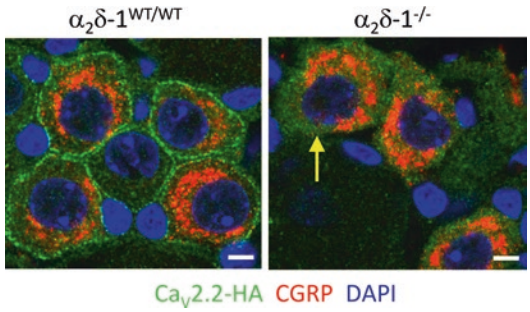

Fig. 4 Effect of $\alpha_2\delta$-1 knockout on $Ca_V2.2$-HA distribution in mouse DRG neurons. DRG sections, showing immunostaining for HA (green) in $Ca_V2.2\_HA^{KI/KI}$ DRGs, co-stained with CGRP (red). Nuclei are stained with 4′,6-diamidino-2-phenylindole (DAPI, blue). Left: $\alpha_2\delta$-$1^{+/+}$ DRGs, showing $Ca_V2.2$ presence at the cell surface; right: $\alpha_2\delta$-$1^{-/-}$ DRGs, showing no $Ca_V2.2$ at the cell surface (e.g., yellow arrow). Scale bar 5 µm. (Images taken from Nieto-Rostro et al., 2018 under CC-BY 4.0 license)

which is much more pronounced for $\alpha_2\delta$-1/2 than for $\alpha_2\delta$-3 (Meyer & Dolphin, 2021), with no effect on endocytosis (Cassidy et al., 2014). Ca_V channels with mutations in their selectivity filter, such that they do not conduct Ca^{2+}, show defective trafficking (Meyer et al., 2019). However, the trafficking of these permeation-defective channels is still enhanced by $\alpha_2\delta$-1 (Meyer et al., 2019), indicating that $\alpha_2\delta$ subunits do not represent a checkpoint such that only functional Ca_V channels respond to them with increased trafficking.

The development of a knock-in mouse containing an epitope tag in endogenous $Ca_V2.2$ has allowed the effect of knockout of $\alpha_2\delta$-1 to be investigated on the distribution of endogenous $Ca_V2.2$ (Nieto-Rostro et al., 2018). This study showed that $Ca_V2.2$ is strongly expressed on the cell surface of particular types of nociceptive DRG neurons and in their presynaptic terminals in the dorsal horn (Fig. 4). Knockout of $\alpha_2\delta$-1 dramatically reduced the cell surface expression of $Ca_V2.2$ and presynaptic distribution (Fig. 4) (Nieto-Rostro et al., 2018), confirming the importance of $\alpha_2\delta$-1 proteins in $Ca_V2.2$ trafficking within the pain pathway.

In contrast, the Ca_V1 channels appear to show a smaller trafficking response to $\alpha_2\delta$ subunits than the Ca_V2 channels, which may reflect their greater membrane stability in cardiac and skeletal muscle. For example, knockdown of $\alpha_2\delta$-1 in muscle cells did not affect targeting and membrane expression of $Ca_V1.1$ and $Ca_V1.2$ and only modestly decreased $Ca_V1.2$ current density, while it strongly affected activation and inactivation kinetics (Obermair et al., 2005; Tuluc et al., 2007).

Cachd1 Function

The roles of the Cache domains in $\alpha_2\delta$ subunits remain unclear. Relevant to this, the $\alpha_2\delta$-like Cachd1 protein was found to produce an increase in $Ca_V2.2$ but not $Ca_V2.1$ currents (Dahimene et al., 2018), and quite contrary to $\alpha_2\delta$ subunits, Cachd1 was also found to produce an increase in Ca_V3 T-type channel currents (Cottrell et al., 2018). The basis for this differential selectivity remains unclear and might relate to specific splice variants, or interactions with specific extracellular elements of the different Ca_V channels. Although Cachd1 was found to increase $Ca_V2.2$ currents, this was to a much smaller extent than observed with $\alpha_2\delta$ subunits (Dahimene et al., 2018), and it also increased $Ca_V2.2$ cell surface expression in parallel (Dahimene et al., 2018). However, unlike $\alpha_2\delta$-1, the interaction with Cachd1 did not require its imperfect VWA domain interacting with the first extracellular loop of the $Ca_V2.2$ α_1 subunit (Dahimene et al., 2018). Therefore, for $Ca_V2.2$, it is likely to involve other interactions, for example, between the Cache domains of Cachd1 and extracellular domains of the α_1 subunit.

Synaptic Functions of $\alpha_2\delta$ Proteins Beyond Their Role as Calcium Channel Subunits

Importance of $\alpha_2\delta$ Proteins in Neuronal and Synaptic Functions

Several studies implicate an involvement of $\alpha_2\delta$ proteins in neuronal and synaptic functions, which partly go beyond their role as Ca_V channel subunits (Chen et al., 2018; Eroglu et al., 2009;

Geisler et al., 2019; Kurshan et al., 2009; Schöpf et al., 2021). Most importantly, $\alpha_2\delta$ proteins have emerged as critical regulators of synapse formation and differentiation in central nervous system neurons (Eroglu et al., 2009; Schöpf et al., 2021) and expression of specific $\alpha_2\delta$ isoforms during distinct developmental phases may shape the structural and functional neuronal network connectivity (Bikbaev et al., 2020). While postsynaptically located $\alpha_2\delta$-1 subunits have been found to be relevant for basic neuronal differentiation and for modulating postsynaptic signaling (Eroglu et al., 2009), for example, by serving as a receptor for the astrocyte-secreted and synaptogenic thrombospondins (TSPs), presynaptic $\alpha_2\delta$ proteins serve dual purposes: on one hand, they regulate the abundance of presynaptic calcium channels and hence can directly regulate the efficacy of synaptic transmission (Ferron et al., 2018; Hoppa et al., 2012; Schöpf et al., 2021). On the other hand, they regulate synapse formation, differentiation, and the trans-synaptic recruitment of postsynaptic receptors (Fell et al., 2016; Geisler et al., 2019; Schöpf et al., 2021).

Postsynaptic Functions of $\alpha_2\delta$-1

In retinal ganglion cells the interaction of postsynaptic $\alpha_2\delta$-1 and astrocyte-secreted thrombospondin-1 (TSP-1) mediates excitatory synapse formation in a mechanism, which is independent of the presence and function of α_1 subunits (Eroglu et al., 2009) but involves the recruitment of NMDA receptors to the postsynaptic membrane (Risher et al., 2018). A role in synapse formation is further supported by impaired excitatory synaptogenesis and spine morphology in conditional (Risher et al., 2018) and constitutive (Bikbaev et al., 2020) $\alpha_2\delta$-1 knockout mice. TSP-4 was also identified as an $\alpha_2\delta$-1 ligand (Eroglu et al., 2009), although the interaction was subsequently found to be weak (El-Awaad et al., 2019; Lana et al., 2016), and less evident than for another known TSP ligand, low-density lipoprotein receptor-related protein 1 (LRP1) (Lana et al., 2016). However, and in contrast to TSP-1, this interaction likely occurs with presynaptic $\alpha_2\delta$ subunits (Yu et al., 2018).

Presynaptic and Trans-synaptic Roles of $\alpha_2\delta$ Proteins

We recently showed that in cultured hippocampal neurons presynaptic triple knockout/knockdown of all brain $\alpha_2\delta$ isoforms severely compromised synapse formation and pre- and postsynaptic differentiation (Fig. 5) (Schöpf et al., 2021). This striking phenotype could be rescued by the expression of each individual $\alpha_2\delta$ isoform, suggesting a surprisingly redundant presynaptic and trans-synaptic role in this critical neuronal function. Trans-synaptic signaling via $\alpha_2\delta$ proteins is supported by other recent observations. Expression of an $\alpha_2\delta$-2 splice variant lacking exon 23 (splice site C, see above) in hippocampal neurons is sufficient to trigger the aberrant wiring of presynaptic excitatory (glutamatergic) axons to inhibitory (GABAergic) postsynaptic sites (Fig. 6). Furthermore, this $\alpha_2\delta$-2 splice variant, when expressed in presynaptic nerve terminals, regulates postsynaptic $GABA_A$ receptor ($GABA_AR$) abundance, both in aberrantly wired synapses as well as normally wired inhibitory synapses (Geisler et al., 2019). Also, in inner hair cell synapses of the cochlea, $\alpha_2\delta$-2 is necessary for the proper spatial alignment of presynaptic L-type $Ca_V1.3$ calcium channels and postsynaptic AMPA receptors (Fell et al., 2016). The $\alpha_2\delta$-2 isoform is also involved in modulating axonal regeneration after injury and establishing neuronal circuits (Tedeschi et al., 2016). Synaptic functions of $\alpha_2\delta$-3 so far have mainly been addressed in invertebrate model systems, where it was involved in regulating the size and morphology of motoneuron terminals (Caylor et al., 2013; Kurshan et al., 2009). A role for $\alpha_2\delta$-3 in regulating presynaptic differentiation has also been confirmed in vertebrates, by studying the consequences of $\alpha_2\delta$-3 knockout on auditory nerve fibers (Pirone et al., 2014) and the expression of $\alpha_2\delta$-3 in GABAergic neurons (Geisler

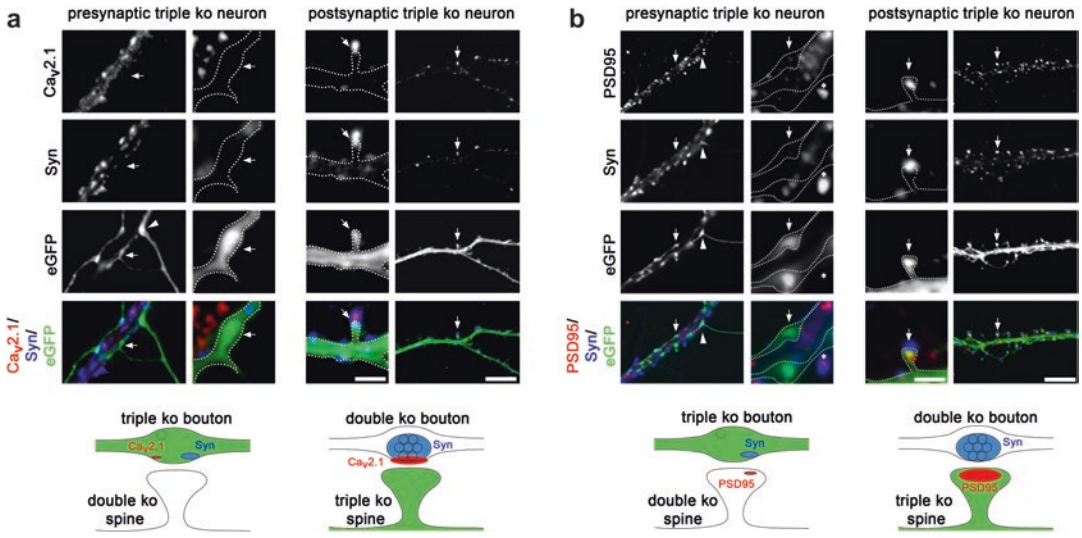

Fig. 5 Presynaptic $\alpha_2\delta$ subunits mediate excitatory synapse formation and trans-synaptic differentiation. Immunofluorescence labeling of eGFP-labeled axons with presynaptic boutons (**a** and **b**, left panels, arrows) and postsynaptic dendrites with dendritic spines (**a** and **b**, right panels, arrows) from presynaptic or postsynaptic $\alpha_2\delta$ triple knockout/knockdown hippocampal neurons. The sketches summarize the observed labelling patterns. (Images taken from Figure 4 of Schöpf et al., 2021 under a CC BY 4.0 license). Scale bars, 2 µm (selection) and 8 µm (overview). (**a**) Presynaptic triple knockout/knockdown of $\alpha_2\delta$-1, $\alpha_2\delta$-2, and $\alpha_2\delta$-3 results in failed calcium channel and synapsin clustering (left panel, arrows and sketch). In contrast, dendritic spines opposite presynaptic boutons containing Cav2.1 and synapsin clusters develop normal when all $\alpha_2\delta$ subunits have been knocked out/down in the postsynaptic neuron (right panel, arrows and sketch). (**b**) Presynaptic triple knockout/knockdown of $\alpha_2\delta$-1, $\alpha_2\delta$-2, and $\alpha_2\delta$-3 also affects postsynaptic differentiation as seen by missing PSD95 clustering (left panel, arrows and sketch) opposite defective presynaptic terminals. In contrast, postsynaptic $\alpha_2\delta$ subunit triple knockout/knockdown does not affect PSD95 clustering opposite presynaptic terminals containing Cav2.1 and synapsin clusters (right panel, arrows and sketch)

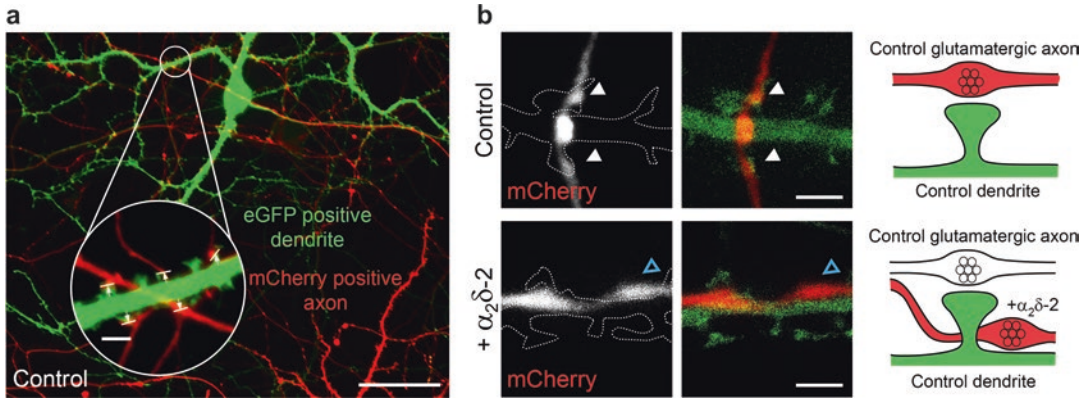

Fig. 6 Presynaptic $\alpha_2\delta$-2 induces aberrant wiring of excitatory synapses. (**a**) Experimental setup to analyze the position of synapses on postsynaptic dendrites of excitatory cultured hippocampal neurons: Presynaptic control or $\alpha_2\delta$-2 expressing neurons were labeled with mCherry (red) and postsynaptic neurons with eGFP (green). The magnified inset shows the contact of presynaptic axonal boutons (mCherry) with postsynaptic dendritic spines (eGFP), as expected for excitatory spine synapses. Scale bars, 50 µm (overview), 3 µm (inset). (**b**) Super-resolution gSTED microscopy confirms the preferential location of excitatory synapses on dendritic spines of excitatory glutamatergic neurons (white arrowheads and sketch). Strikingly, presynaptic axons expressing $\alpha_2\delta$-2_ΔE23 aberrantly wire to postsynaptic sites along dendritic shafts (blue arrowheads and sketch), a position typically observed for GABAergic synapses. Scale bars, 2 µm. (Images taken from Figure 11 of Geisler et al., 2019 under a CC BY 4.0 license)

et al., 2019). In the context of $\alpha_2\delta$ functions independent of the calcium channel complex, it is noteworthy that a quantitative study of the neuronal Ca_V2 channel proteome suggested a considerably weaker association of $\alpha_2\delta$ with α_1 subunits when compared to β subunits, although this was dependent on the detergent used for membrane solubilization (Müller et al., 2010). Furthermore, α_1 and $\alpha_2\delta$ subunit interactions in the neuronal membrane are likely dynamic and association may only be transient, as suggested by single-molecule live-cell imaging (Brockhaus et al., 2018; Schneider et al., 2015; Voigt et al., 2016). In retinal photoreceptor cells, where the L-type channel $Ca_V1.4$ serves as the main presynaptic channel of ribbon synapses, $\alpha_2\delta$-4 regulates functional membrane expression of α_1 subunits and synaptic transmission of rods (Wang et al., 2017) and cones (Kerov et al., 2018; Schlegel et al., 2019). In line with a trans-synaptic role of $\alpha_2\delta$ proteins, knockout $\alpha_2\delta$-4 affects not only presynaptic structure but also postsynaptic receptor clustering (Wang et al., 2017). Finally, the well-characterized mechanism of $\alpha_2\delta$-1 in neuropathic pain and the development of hyperalgesia are also mediated by presynaptic mechanisms (Bauer et al., 2009; Nieto-Rostro et al., 2018; Yu et al., 2018).

Diseases Associated with $\alpha_2\delta$ Subunits

It is evident from the above that $\alpha_2\delta$ proteins are abundantly expressed in various organs and particularly in the brain, and that they are involved in critical calcium channel-dependent as well as-independent functions. Hence, it is not surprising that calcium channel dysfunctions are associated with a large variety of disorders. Over recent years an increasing number of disorders in humans have been linked to the genes encoding $\alpha_2\delta$ subunits (reviewed in Ablinger et al., 2020), however, so far detailed insights into pathophysiological mechanisms are only beginning to emerge.

Ducky Mice and Human Mutations of the $\alpha_2\delta$-2 Gene

A number of recessive mutations in *Cacna2d2* underlie the cerebellar ataxia and epilepsy phenotypes seen in spontaneously arising mouse mutants *ducky*, *ducky*^{2J}, and *entla*, as well as engineered *Cacna2d2* knockout mice (Barclay et al., 2001; Brill et al., 2004; Brodbeck et al., 2002; Donato et al., 2006; Geisler et al., 2021; Ivanov et al., 2004). Furthermore, mutations in human *CACNA2D2* (located on chromosome 3p21.31) are associated with recessive epileptic encephalopathy and mental retardation (Edvardson et al., 2013; Pippucci et al., 2013; Punetha et al., 2019) and may be also be linked to schizophrenia (Rodríguez-López et al., 2018).

Mutations in the $\alpha_2\delta$-1 Gene Associated with Cardiac Phenotypes

CACNA2D1 (located on chromosome 7q21.11) mutations have been reported to be associated with cardiac dysfunction, including short QT syndrome (Templin et al., 2011) and Brugada syndrome (Burashnikov et al., 2010), although the effects of these mutations measured experimentally are rather inconsistent (Bourdin et al., 2015). In agreement, homozygous *Cacna2d1* knockout causes a mild cardiac phenotype in mice (Fuller-Bicer et al., 2009) and additionally increases the susceptibility for diabetes (Mastrolia et al., 2017). However, several cases have been identified of epileptic encephalopathy involving copy number variants that usually contain multiple genes including *CACNA2D1* (Mefford et al., 2011; Vergult et al., 2015). A recent study also found $\alpha_2\delta$-1 was a target for autoimmune encephalitis (Lee et al., 2021), and another study also showed the presence of $\alpha_2\delta$-1 auto-antibodies in cases of amyotrophic lateral sclerosis associated with type 2 diabetes (Shi et al., 2019).

Neuropathic Injury and the Role of $\alpha_2\delta$-1

Peripheral sensory nerve injury in a variety of rodent models, including spinal nerve injury and administration of chemotherapeutic drugs, results in an increase of $\alpha_2\delta$-1 mRNA in damaged DRG neurons (Bauer et al., 2009; Lana et al., 2014; Newton et al., 2001; Wang et al., 2002; Xiao et al., 2007). This gives rise to a corresponding increase of $\alpha_2\delta$-1 protein within the injured DRG cell bodies, and also in their primary afferent terminals (Bauer et al., 2009; Luo et al., 2001). Furthermore, there was a differential upregulation of a splice variant of $\alpha_2\delta$-1 that shows a lower affinity for ^{3}H-gabapentin (Lana et al., 2014). In agreement with these results, *Cacna2d1* knockout mice showed reduced sensitivity to mechanical stimulation and delayed onset of neuropathic mechanical hypersensitivity following peripheral nerve injury (Patel et al., 2013). Furthermore, $\alpha_2\delta$-1 overexpressing mice have been generated, and they show increased baseline response to painful stimuli (Li et al., 2006).

Disorders Associated with $\alpha_2\delta$-3

From knockout models of *Cacna2d3*, a clear role for $\alpha_2\delta$-3 in hearing has been identified (Pirone et al., 2014), but no related human mutations in *CACNA2D3* (located on chromosome 3p21.1) have yet been reported. However, independent studies qualify *CACNA2D3* as risk gene for autism spectrum disorders (see below). Furthermore, a genome-wide Drosophila RNAi screen for heat nociception identified *Cacna2d3* as a pain-related gene, which was supported by the phenotype of $\alpha_2\delta$-3 knockout mice and human single-nucleotide polymorphisms (SNPs) associated with pain conditions, although potential human disease mechanisms are still unknown (Neely et al., 2010).

Disorders Associated with $\alpha_2\delta$-4

Mutations in both mouse and human *CACNA2D4* (located on chromosome 12p13.33 in humans) cause dysfunction of photoreceptors, resulting in certain forms of night blindness (Wycisk et al., 2006a, b).

Psychiatric Disorders

An interesting common feature of $\alpha_2\delta$ subunits is that over recent years SNPs or genomic variations in all *CACNA2D* ($\alpha_2\delta$ subunit) genes have been linked to a spectrum of psychiatric disorders (Ablinger et al., 2020; Consortium, 2013). Indeed, SNPs in *CACNA2D2* and *CACNA2D4* exhibited statistically significant associations with disease, across multiple psychiatric conditions. More recently, an excess of several rare disruptive mutations in *CACNA2D1, CACNA2D2, CACNA2D3, and CACNA2D4* were observed in cases of schizophrenia (Purcell et al., 2014), as reviewed recently (Ablinger et al., 2020; Heyes et al., 2015). Furthermore, several mutations in *CACNA2D3*, including a splice site mutation (Iossifov et al., 2012), are potentially linked to autism spectrum disorders (De Rubeis et al., 2014; Guo et al., 2018). Similarly in *CACNA2D1* a de novo mutation has been linked to autism (Iossifov et al., 2014), and a recent exome sequencing study suggests *CACNA2D1* as candidate risk gene for neurodevelopmental disorders (Valentino et al., 2021). Furthermore, a partial *CACNA2D4* deletion has been associated with rare cases of late onset bipolar disorder (Van Den Bossche et al., 2012).

Pharmacology Involving $\alpha_2\delta$ Subunits

Therapeutic Uses of Gabapentinoids

Gabapentin was first identified as an anti-epileptic drug (Crawford et al., 1987), and then as having efficacy in painful neuropathies (Wiffen et al.,

2005). There are currently three therapeutically available gabapentinoid $\alpha_2\delta$ ligands which are used in chronic neuropathic pain conditions: gabapentin, pregabalin (Field et al., 1999; Li et al., 2011), and mirogabalin (Domon et al., 2018).

Gabapentinoid Drug Binding to $\alpha_2\delta$ Subunits and Potential Mechanisms of Action

The therapeutic target for gabapentin was identified as $\alpha_2\delta$-1 by purifying a brain protein that binds to ^{3}H-gabapentin (Brown & Gee, 1998; Gee et al., 1996). However, gabapentin has very little effect acutely on calcium currents (Hendrich et al., 2008; Kang et al., 2002; Martin et al., 2002), but chronic incubation with gabapentin for 24–48 h reduces calcium currents (Biggs et al., 2014; Hendrich et al., 2008), synaptic transmission (Hendrich et al., 2012; Lempel et al., 2017), and cell surface expression of $\alpha_2\delta$-1, $\alpha_2\delta$-2, and $Ca_V2.2$ (Cassidy et al., 2014; Tran-Van-Minh & Dolphin, 2010).

The binding site for gabapentin was found to include an RRR motif, which is just upstream of the VWA domain (Brown & Gee, 1998; Wang et al., 1999). This is present in $\alpha_2\delta$-1/2 but not the other $\alpha_2\delta$ subunits which do not bind gabapentin (Gong et al., 2001; Marais et al., 2001). If the third R is mutated to A in $\alpha_2\delta$-1 or $\alpha_2\delta$-2, this reduces the affinity of gabapentinoid binding and $\alpha_2\delta$ function (Field et al., 2006; Hendrich et al., 2008; Tran-Van-Minh & Dolphin, 2010; Wang et al., 1999). It also reduces the response to gabapentinoids in chronic neuropathic pain models (Field et al., 2006) and in experimental epilepsy and anxiety models (Lotarski et al., 2011, 2014; Taylor et al., 2007). The skeletal muscle splice variant of $\alpha_2\delta$-1 (+A + BΔC) bound to ^{3}H-gabapentin with high affinity (Lana et al., 2014), in agreement with data from rat skeletal muscle $\alpha_2\delta$-1 (Gee et al., 1996). Thus, the lack of effect of gabapentin on skeletal muscle function is not a result of its inability to bind to the skeletal muscle $\alpha_2\delta$-1 isoform and could relate instead to the high stability of the calcium channel complex in skeletal muscle.

The First Double Cache Domain in $\alpha_2\delta$-1 Has Structural Homology to a Universal Amino Acid Binding Domain

A ubiquitous extracellular double Cache domain has been identified in bacterial chemoreceptors and chemotransducers that contains a simple amino acid recognition motif. In bacteria and archaea this has been found to exclusively bind a variety of amino acids, whose identity and binding affinity depends on the exact residues in the binding pocket (Gumerov et al., 2022). In eukaryotes the same motif is found only in $\alpha_2\delta$ proteins and Cachd1, and in $\alpha_2\delta$-1 the first double Cache domain (consisting of Cache 1 and Cache 2, Fig. 3a) contains the binding site for gabapentin (Gumerov et al., 2022). It has previously been shown that $\alpha_2\delta$-1 also binds leucine and isoleucine, and these compete with gabapentin (Brown et al., 1998). Whether endogenous amino acids have a physiological role in binding to $\alpha_2\delta$ proteins, for example, as positive or negative modulators of trafficking, is yet unknown.

Effect of $\alpha_2\delta$ Subunits on Ziconotide Binding

The presence of $\alpha_2\delta$ proteins was found to reduce the on-rate and equilibrium inhibition of the $Ca_V2.2$ channel blocking ω-conotoxins (Mould et al., 2004). This study included ω-conotoxin MVIIA or ziconotide, which is licensed for use in neuropathic pain conditions. A recent structural study has identified the mechanism for $\alpha_2\delta$ interfering with the drug binding site, as ziconotide alters the orientation of $\alpha_2\delta$-1 with respect to the channel (Gao et al., 2021).

Other Interactions of $\alpha_2\delta$ Proteins

The $\alpha_2\delta$s, being completely extracellular, and with structural domain similarities to other extracellular matrix and cell adhesion proteins (Whittaker & Hynes, 2002), are likely to have interactions with other proteins. For example,

they may be associated with proteins involved in ion channel clustering. Indeed they have also variously been shown to either directly interact with or influence the function of, several other proteins, including the trafficking and endocytosis protein LRP1 (Kadurin et al., 2017), the extracellular matrix proteins, α-neurexins (Brockhaus et al., 2018; Tong et al., 2017), and other ion channels, such as BK channels (Zhang et al., 2018). Interactions of $\alpha_2\delta$-1 with NMDA receptors (Chen et al., 2018) and certain AMPA receptors (Li et al., 2021) have also been identified. The interaction of $\alpha_2\delta$-1 with these glutamate receptors was shown to involve the extreme C-terminus of $\alpha_2\delta$-1 which would normally be cleaved off during the process of GPI anchoring in the ER (Davies et al., 2010; Guizzunti & Zurzolo, 2014); thus, any interaction must either occur in the ER and prevent the formation of the GPI-anchored $\alpha_2\delta$-1, or the interaction occurs with the cleaved C-terminal GPI signal peptide, which has been shown to be rapidly degraded for other GPI-anchored proteins (Guizzunti & Zurzolo, 2014). This topic was recently reviewed (Dolphin, 2018). Generally, such potential interactions may also serve to co-locate multiple ion channels with neurotransmitter receptors and other signaling proteins within synaptic structures.

Summary and Outlook

Over the last 20 years, our appreciation of the Ca_V $\alpha_2\delta$ subunits has changed considerably: from a purely auxiliary channel subunit, which modulates some biophysical channel properties, to a ubiquitous, albeit enigmatic, signaling protein, which serves as an important drug target and regulates synaptic function. This remarkable evolution is based on several scientific developments: first, the study of calcium channels and hence $\alpha_2\delta$ subunits was conferred progressively from heterologous expression systems into native, differentiated cells and tissues. Second, knockout and mutant mouse models became available, which highlighted the functional importance of $\alpha_2\delta$ proteins in distinct tissues and physiological functions. Third and particularly owing to the increasingly employed OMICs technologies, our insights into disease associations are expanding at an accelerated speed. Because $\alpha_2\delta$ proteins have been linked to a variety of diseases, ranging from hormone secretion to neuropsychiatric disorders (and many new disease associations may be identified in future years), pharmacological targeting of $\alpha_2\delta$ proteins bears a tremendous therapeutic potential. Indeed, gabapentin is used as an anti-epileptic drug, and all gabapentinoids are widely prescribed for treating neuropathic pain. However, before the full theoretical therapeutic potential can be exploited, a number of questions need to be addressed:

1. Several studies have identified and proposed $\alpha_2\delta$ isoform and even splice variant-specific functions and disease associations. However, the physiological importance of the functional redundancy between different $\alpha_2\delta$ isoforms is not yet understood. In this context it is noteworthy that the synaptic phenotype in a presynaptic $\alpha_2\delta$ triple knockout/knockdown model can be rescued by the expression of each brain $\alpha_2\delta$ isoform (Schöpf et al., 2021).
2. As discussed above, recent studies propose calcium channel-independent functions of $\alpha_2\delta$ proteins. However, any experimental condition or pathophysiological mechanism affecting the expression and function of $\alpha_2\delta$ subunits will also affect calcium channels; hence, the definitive distinction between calcium channel-dependent and -independent functions represents an experimental challenge. Yet, a thorough understanding is a prerequisite for novel therapeutic concepts, for example, for targeting trans-synaptic functions without altering/inhibiting calcium channels.
3. On one hand, OMICs technologies continue to identify novel disease associations for all $\alpha_2\delta$ isoforms (reviewed in Ablinger et al., 2020). On the other hand, detailed insights into the pathophysiological mechanisms are

limited to a few examples, such as the key role of $\alpha_2\delta$-1 upregulation in neuropathic pain (reviewed in Dolphin, 2016). The experimental challenge for future years thus lies in elucidating the mechanisms linking the $\alpha_2\delta$ proteins to disease, both in terms of calcium channel-dependent and -independent functions.

4. Our current understanding about the $\alpha_2\delta$ isoform and splice variant-specific expression, particularly during development and disease, is limited to tissues and brain regions. Investigating cell-type-specific expression patterns is another prerequisite for ultimately understanding specific functions, such as the establishment of network connectivity (Bikbaev et al., 2020). For example, in brain expression of the retinal $\alpha_2\delta$-4 isoform is extremely low, basically negligible (Schlick et al., 2010). Nevertheless, $\alpha_2\delta$-4 expression is strongly upregulated during development and disease (Schlick et al., 2010; van Loo et al., 2019). In theory this may be related to an important role of $\alpha_2\delta$-4 in a single and rare neuron type. Hence, establishing conditional $\alpha_2\delta$ knockout mouse models may provide a first step into studying the cell-type-specific expression and function.
5. As discussed above, $\alpha_2\delta$ proteins are emerging as critical regulators of synapse formation, trans-synaptic signaling, and axonal/synaptic wiring. Future novel therapeutic paradigms affecting the expression and function of $\alpha_2\delta$ thus may have the potential to modulate synaptic wiring during and after development (Bikbaev et al., 2020; Geisler et al., 2019; Veroniki et al., 2017). Hence, the ethical basis for potentially interfering with neurodevelopment requires a discussion between scientists and affected individuals, for example, people with autism (Sanderson, 2021).
6. Finally, considering the newly discovered synaptic roles of $\alpha_2\delta$, the consequences on human behavior (e.g., learning) of the extremely widespread used gabapentinoid drugs need to be more thoroughly investigated. Nevertheless, it should be noted that a meta-analysis of adverse effects of antiepileptic drugs did not detect negative effects of gabapentin on cognitive development of children exposed during pregnancy or breastfeeding (Veroniki et al., 2017). Direct effects on synaptic transmission, synaptic wiring, and postsynaptic receptor clustering may ultimately qualify gabapentinoid drugs to be classified as a psychiatric medication. Because these drugs are predominantly prescribed for chronic pain conditions, they also bear a considerable abuse potential, which so far has not been sufficiently investigated (Goins et al., 2021).

Taken together, understanding the multiple functions of $\alpha_2\delta$ proteins, which are ubiquitously expressed in excitable cells, has come a long way. Future research efforts will be aimed at elucidating pathophysiological mechanisms of associated disorders and may lead to novel therapeutic paradigms.

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