Chapter 15
Sensory Pathway Modulation by Calcium Channel $\alpha_2\delta_1$ Subunit

Chunyi Zhou and Z. David Luo

Abstract Voltage-gated calcium channels (VGCC) are importantly involved in modulation of pathophysiological functions, including the transduction of nociceptive and non-nociceptive signals. As an auxiliary subunit of VGCC, the $\alpha_2\delta$ ($Ca_v\alpha_2\delta$) subunit plays critical roles in modulating VGCC expression and functions such as regulations of VGCC trafficking, kinetics of voltage-dependent activation and inactivation. $Ca_v\alpha_2\delta$ also modulates neuronal and synaptic functions through both VGCC-dependent and independent mechanisms. Among $Ca_v\alpha_2\delta_{1-4}$ subunits, $Ca_v\alpha_2\delta_1$ subunit is implicated in pain processing because (1) its upregulation in neuropathic pain models is shown to play a critical role in the onset and maintenance of pain states; (2) its upregulation in sensory neurons leads to dorsal spinal cord neuron sensitization; (3) it is the receptor for gabapentinoids that can normalize activity of sensitized dorsal spinal cord neurons, and have anti-neuropathic pain properties in animal models and patients. In this chapter, we briefly review the regulation of $Ca_v\alpha_2\delta$ and its functional contribution to pathophysiological conditions with a main focus on pain transduction and processing. Underlying mechanisms related to $Ca_v\alpha_2\delta_1$ contributions to pain processing and the therapeutic effects of gabapentinoids are also discussed.

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15.1 Introduction

Ca^{2+} is one of the most important and abundant elements in the body. Membrane depolarization activates voltage-gated calcium channels (VGCC), and causes Ca^{2+} influx, which in turn acts as a second messenger to trigger various intracellular events including enzyme activation, neurotransmitter and hormone release, cell-cell communication, contraction of different kinds of contractile cells, gene expression, cell division, migration and death.

The purified VGCC complex is composed of four subunits, primary channel-forming α_1, auxiliary β, $\alpha_2\delta$, and in some tissue, γ subunits (Takahashi and Catterall 1987; Takahashi et al. 1987; Ertel et al. 2000). Four homologous domains of the α_1 subunit form a Ca^{2+} selective pore. The intracellular β/γ subunits and transmembrane $\alpha_2\delta$ subunit modulate the trafficking and functioning of the VGCC (Felix 1999; Hofmann et al. 1999; Catterall 2000).

Based on membrane potentials required for activation, VGCC were initially divided into high-voltage-activated (HVA) and low-voltage-activated (LVA) channels (Fedulova et al. 1985), then further classified as L-, N- P/Q-, R- and T-type based on their distinct biophysical and pharmacological properties (Nowycky et al. 1985; Dolphin 2006). T-type VGCC have a low-voltage activation threshold, can be activated at the resting membrane potential, thus, contributing to pacemaker activity in excitable cells. Other VGCC have high-voltage activation thresholds, and can be activated at more depolarized membrane potentials. Functional L-, N-, P/Q- and R-type VGCC comprise the principle α_1 subunit, as well as the β and $\alpha_2\delta$ auxiliary subunits in a 1:1:1 stoichiometry. T-type VGCC, on the other hand, appear to require only α_1 subunit for correct function (Bean 1989; Felix 1999; Hofmann et al. 1999; Catterall 2000; Ertel et al. 2000; Dolphin 2006).

The development of selective $Ca_v\alpha_2\delta$ ligands, the gabapentinoids including gabapentin and pregabalin, not only provides us with novel therapeutic agents for neuropathic pain management, but also allows more extensive study of the function of $Ca_v\alpha_2\delta$ at the cellular and molecular level. It is known that $Ca_v\alpha_2\delta$ plays a role in regulating VGCC trafficking to the plasma membrane (Gurnett et al. 1997; Bernstein and Jones 2007), and fine-tuning channel gating properties (Mori et al. 1991; Singer et al. 1991; Klugbauer et al. 1999, 2003; Gao et al. 2000; Davies et al. 2006). There is also emerging evidence suggesting that $Ca_v\alpha_2\delta$ may have functions independent of VGCC. After a brief overview of VGCC subunits, this chapter mainly focuses on structure, cellular/molecular biology and functions of the $Ca_v\alpha_2\delta$ subunit, the mechanisms underlying the action of $Ca_v\alpha_2\delta_1$ proteins on synaptic calcium channel activities, excitatory synaptogenesis that may underlie the mechanism of gabapentinoids in pain modulation.

15.2 Calcium Channel Subunits

The channel forming α_1 subunit ($Ca_v\alpha_1$, 175 kDa) is the principle subunit of VGCC. In mammalian cells, there are ten genes encoding $Ca_v\alpha_1$. Based on amino acid sequence similarity, the $Ca_v\alpha_1$ subunit can be divided into three subfamilies: Ca_v1, Ca_v2, and Ca_v3 (Catterall 2000; Ertel et al. 2000; Arikkath and Campbell 2003), which are classified as L-type ($Ca_v1.1$, $Ca_v1.2$, $Ca_v1.3$, $Ca_v1.4$), P/Q-type ($Ca_v2.1$), N-type ($Ca_v2.2$), R-type ($Ca_v2.3$), and T-type ($Ca_v3.1$, $Ca_v3.2$, $Ca_v3.3$) VGCC based on their pharmacology, electrophysiological properties, as well as physiological functions (Hofmann et al. 1999; Catterall 2000; Striessnig and Koschak 2008). Each $Ca_v\alpha_1$ contains four homologous domains connected by cytoplasmic loops. Each domain has six transmembrane segments. There is a pore-forming loop (P-loop) between S5 and S6, which contains a highly conserved, negatively charged amino acid, either glutamate or aspartate, forming a signature locus that is essential for Ca^{2+} selection and conduction (Kim et al. 1993; Kuo and Hess 1993). The S4 segment of each domain that contains positively charged amino acids serves as the voltage sensor for activation and initiation of conformational changes that open the pore. These structural features contribute to VGCC gating, ion selectivity, and permeation. $Ca_v\alpha_1$ also contains the interaction sites for other subunits, VGCC blockers and activators. Although $Ca_v\alpha_1$ subunits are responsible for the physiological and pharmacological properties of calcium channels, the trafficking and functioning of different types of VGCC require the auxiliary β and $\alpha_2\delta$ subunits (Ertel et al. 2000; Arikkath and Campbell 2003; Buraei and Yang 2010).

The β subunit ($Ca_v\beta$, 54 kDa) is an intracellular hydrophilic protein. There are four different types of $Ca_v\beta$ ($Cav\beta_{1-4}$), each with splice variants, encoded by four distinct genes. All four $Ca_v\beta$ share a common central core, whereas their N- and C-termini differ significantly. All four $Ca_v\beta$ dramatically enhance calcium channel currents when they are coexpressed along with the $Ca_v\alpha_1$ subunit in heterologous expression systems. $Ca_v\beta$ can also modulate the voltage-dependence, kinetics of activation and inactivation without affecting ion permeation (Obermair et al. 2008; Dolphin 2009; Karunasekara et al. 2009). $Ca_v\beta$ interacts with $Ca_v\alpha_1$ mainly through the β-interaction domain (BID) that binds with high-affinity to the α-interaction domain (AID) in the cytoplasmic loop of $Ca_v\alpha_1$ connecting the first two homologous repeats (De Waard et al. 1995; Witcher et al. 1995; Chen et al. 2004).

The γ subunit ($Ca_v\gamma$, 30 kDa) is an intracellular hydrophilic protein. There are eight different genes encoding $Ca_v\gamma$ subunits ($Ca_v\gamma_{1-8}$). Various $Ca_v\gamma$ subunits have been shown to affect kinetics and voltage-dependent gating of VGCC (Kang and Campbell 2003; Chen et al. 2007). $Ca_v\gamma_1$ was first cloned from muscle VGCC (Jay et al. 1990). Coexpression of $Ca_v\gamma$ subunit with L-type calcium channel subunits modulates Ca^{2+} peak current, activation and inactivation kinetics. This has been confirmed by subsequent studies in $Ca_v\gamma_1$ knockout mice (Arikkath et al. 2003), which show increased Ca^{2+} peak currents and altered inactivation kinetics compared with their age and sex matched wild type littermates (Freise et al. 2000).

In stagazer mutant mice, $Ca_v\gamma_2$ subunit levels are significantly reduced, and this change shifts calcium channel inactivation to more negative potentials. This deficit accounts for the distinctive phenotype, including head-tossing and ataxic gait (Letts et al. 1998).

All Ca_v1 and Ca_v2 channels contain transmembrane auxiliary $Ca_v\alpha_2\delta$ subunits (Felix 1999; Dolphin 2009). There are four subfamilies of $Ca_v\alpha_2\delta$ subunits ($Ca_v\alpha_2\delta_{1-4}$), each encoded by a unique gene, and the α_2 (143 kDa) and δ (24–27 kDa) peptides are cleaved then linked by disulfide bounds post-translationally (Felix 1999). When co-expressed along with $Ca_v\alpha_1$ and $Ca_v\beta$ subunits of Ca_v1 or Ca_v2 channels in heterologous expression systems, $Ca_v\alpha_2\delta$ subunits can dramatically increase calcium channel currents (Mori et al. 1991; Singer et al. 1991; Klugbauer et al. 1999, 2003; Gao et al. 2000; Davies et al. 2006). The enhancement is associated with the increased trafficking and retention of $Ca_v\alpha_1$ to the plasma membrane (Gurnett et al. 1997; Canti et al. 2005; Bernstein and Jones 2007). The systemic tissue distribution of $Ca_v\alpha_2\delta$ subunits has been analyzed at the mRNA and protein levels by different laboratories (Klugbauer et al. 1999; Hobom et al. 2000; Gong et al. 2001; Marais et al. 2001). $Ca_v\alpha_2\delta_1$ is abundantly expressed in excitable tissues such as the brain, heart, and muscles. $Ca_v\alpha_2\delta_2$ is expressed in various tissues with the highest levels in brain, heart, pancreas, and skeletal muscles. In a more restricted way, $Ca_v\alpha_2\delta_3$ expression levels are high in the brain, but low in the heart and skeletal muscles.

Since $Ca_v\alpha_2\delta_1$ and $Ca_v\alpha_2\delta_2$ are binding sites for gabapentin and pregabalin, which were originally designed as antiepilepsy drugs but have unexpected antineuropathic pain properties (Gee et al. 1996; Field et al. 2006), the contribution of $Ca_v\alpha_2\delta$ subunits, specially the $Ca_v\alpha_2\delta_1$ subunit, to pain processing has been studied extensively in the past decade.

15.3 Structure of $Ca_v\alpha_2\delta$ Subunits

Studies of transmembrane topology of $Ca_v\alpha_2\delta$ subunits have shown that the α_2 peptide is entirely extracellular (Brickley et al. 1995; Gurnett et al. 1996). The δ peptide is originally assumed to be transmembrane through a hydrophobic domain (Brickley et al. 1995; Gurnett et al. 1996). However, Davies et al. have recently reported that the δ peptide is attached to the membrane through a glycosylphosphatidylinositol linker (Davies et al. 2010). Even though $Ca_v\alpha_2\delta_2$ and $Ca_v\alpha_2\delta_3$ share only 56 and 30 % sequence homology with $Ca_v\alpha_2\delta_1$ respectively (Klugbauer et al. 1999), $Ca_v\alpha_2\delta$ subunits share important structure features including a similar transmembrane topology and heavy glycosylation at the extracellular domain (Klugbauer et al. 1999). Gurnett et al. have shown that both the disulfide bond and glycosylation in $Ca_v\alpha_2\delta_1$ play a critical role in enhancing $Ca_v2.1$ currents (Gurnett et al. 1996, 1997). Data from Western blot studies indicate that $Ca_v\alpha_2\delta_1$, $Ca_v\alpha_2\delta_2$, $Ca_v\alpha_2\delta_3$ and $Ca_v\alpha_2\delta_4$ have similar molecular weights: 200 kDa, 190 kDa, 166 kDa and 138 kDa, respectively (Marais et al. 2001). Splicing variants of $Ca_v\alpha_2\delta$ subunits

(five for $Ca_v\alpha_2\delta_1$, and three for $Ca_v\alpha_2\delta_2$), which differ by three to eight amino acid residues, greatly increase the proteome diversity of calcium channels. These splice variants are differentially expressed in cardiac tissue and brain (Klugbauer et al. 1999; Marais et al. 2001).

It has been shown that $Ca_v\alpha_2\delta$ binds to extracellular domains of $Ca_v\alpha_1$ subunit (Felix et al. 1997; Gurnett et al. 1997). One important domain in $Ca_v\alpha_2\delta$ subunits that has been identified through sequence homology is the highly conserved Von Willebrand factor type A domain (VWA, residues 253–430 of $Ca_v\alpha_2\delta_1$, and residues 294–472 of $Ca_v\alpha_2\delta_2$), which is also present in integrins. The VWA domain is extracellular, has binding sites for extracellular matrix proteins, and contains a metal ion-dependent adhesion site (MIDAS) motif (Whittaker and Hynes 2002). Only $Ca_v\alpha_2\delta_1$ and $Ca_v\alpha_2\delta_2$, but not $Ca_v\alpha_2\delta_3$ or $Ca_v\alpha_2\delta_4$, subunits contain the MIDAS motif. Recent findings have suggested that $Ca_v\alpha_2\delta_1$ and $Ca_v\alpha_2\delta_2$ can both interact with $Ca_v\alpha_1$ subunit through the MIDAS motif and undergo an integrin-like switch, therefore, enhancing cell surface trafficking and currents of the calcium channel complex (Canti et al. 2005).

15.4 Pathophysiological Functions of $Ca_v\alpha_2\delta$ Subunit

15.4.1 Regulation of VGCC Expression

Numerous studies indicate that $Ca_v\alpha_2\delta$ subunits can markedly increase normal VGCC surface expression indicated by increased current amplitude in various in vitro heterologous expression systems, including Xenopus oocytes and mammalian cell lines (Mori et al. 1991; Singer et al. 1991; Shistik et al. 1995; Klugbauer et al. 1999, 2003; Gao et al. 2000; Hobom et al. 2000; Barclay et al. 2001; Canti and Dolphin 2003; Field et al. 2006; Davies et al. 2010). Mutation or overexpression of the $Ca_v\alpha_2\delta$ genes in vivo provides us with useful tools to characterize physiological and pathological roles of $Ca_v\alpha_2\delta_2$ in vivo. Spontaneous mutations in the $Ca_v\alpha_2\delta_2$ gene disrupt $Ca_v\alpha_2\delta_2$ expression in *ducky* mice (Brodbeck et al. 2002). Electrophysiological recording data have shown that the loss of $Ca_v\alpha_2\delta_2$ subunit in Purkinje cells of *ducky* mice results in a 35 % decrease in P-type calcium channel current amplitude, but has no effect on single P-type calcium channel conductance (Barclay et al. 2001). These results indicate that loss of $Ca_v\alpha_2\delta_2$ in vivo reduces VGCC surface expression. In contrast, $Ca_v\alpha_2\delta_1$ subunit overexpression in neuronal cells of transgenic mice results in ~60 % larger Ca^{2+} currents in dorsal root ganglion (DRG) sensory neurons, than that from their wild type littermates, which can be blocked by gabapentin in a concentration-dependent manner, supporting that increased $Ca_v\alpha_2\delta_1$ expression leads to elevated VGCC currents in sensory neurons (Li et al. 2006). Since $Ca_v\alpha_2\delta$ subunits do not change single-channel properties of VGCC such as conductance and open probability (Klugbauer et al. 2003), the increase in current amplitude is likely associated with a chaperoning effect of $Ca_v\alpha_2\delta$ subunits on membrane surface VGCC expression.

Once the calcium channel complex reaches the plasma membrane, $Ca_v\alpha_2\delta$ subunits also dramatically alter voltage-dependence and gating kinetics of VGCC. In general, $Ca_v\alpha_2\delta$ subunits shift voltage-dependent activation and inactivation of VGCC to more negative membrane potentials, and accelerate the inactivation kinetics of VGCC (Klugbauer et al. 2003). However, these effects may differ among individual $Ca_v\alpha_2\delta$ subunits (Hobom et al. 2000) and depending on $Ca_v\alpha_2\delta$ levels. In $Ca_v\alpha_2\delta_1$ overexpressing transgenic mice, increased $Ca_v\alpha_2\delta_1$ expression in sensory neurons leads to a shift of voltage-dependent activation to a more negative membrane potential compared with wild type neurons, an increase in voltage-dependence and rate of activation, and a decrease in voltage-dependent deactivation rate (Li et al. 2006). These findings support that elevated $Ca_v\alpha_2\delta_1$ levels also modulate VGCC kinetics.

How does $Ca_v\alpha_2\delta$ enhance calcium channel surface expression? One hypothesis is that a gabapentin binding site in $Ca_v\alpha_2\delta_1$ and $Ca_v\alpha_2\delta_2$ subunits has a chaperoning effect on VGCC as gabapentin intracellularly disrupts the process of $Ca_v\alpha_2\delta$ and Ca_{v2} trafficking, which could be prevented by a single mutation of the gabapentin binding site in $Ca_v\alpha_2\delta_1$ (R217A) and $Ca_v\alpha_2\delta_2$ (R282A) (Heblich et al. 2008). Alternatively, the VWA domain in the $Ca_v\alpha_2$ protein may interact with $Ca_v\alpha_1$ and thus enhance its trafficking to the plasma membrane. Mutations of three key amino acids (D300, S302, and S304) in the MIDAS motif of the VWA domain in $Ca_v\alpha_2\delta_2$ diminish $Ca_V1.2$, $Ca_V2.1$, $Ca_V2.2$ currents, probably through increased intracellular retention of the $Ca_v\alpha_1$ subunit (Canti et al. 2005).

15.4.2 Presynaptic Expression of $Ca_v\alpha_2\delta$ in Terminals of Sensory Neurons

Under normal conditions, $Ca_v\alpha_2\delta$ is expressed in sensory neurons in dorsal root ganglia, then undergoes anterograde transport to the presynaptic terminals in dorsal spinal cord. Dorsal rhyzotomy, which terminates the connection between dorsal root ganglia and dorsal spinal cord, results in about 50 % reduction in dorsal spinal cord $Ca_v\alpha_2\delta_1$ levels (Li et al. 2004). This indicates that, under normal conditions, $Ca_v\alpha_2\delta_1$ in dorsal spinal cord is expressed at both presynaptic and postsynaptic locations. A recent study provides the first direct evidence supporting that $Ca_v\alpha_2\delta_1$ and $Ca_v\alpha_2\delta_2$ increase P/Q VGCC accumulation at presynaptic boutons and enhance vesicle exocytosis and presynaptic function of VGCC (Hoppa et al. 2012).

15.4.3 $Ca_v\alpha_2\delta$ Functions Independent of Calcium Channel Activity

The functions of $Ca_v\alpha_2\delta$ have long been exclusively linked with VGCC. However, recent studies suggest that $Ca_v\alpha_2\delta$ may possess functions independent of their

association with VGCC. Data from a recent study have shown that $Ca_v\alpha_2\delta$ is the receptor for thrombospondin (TSP), an extracellular matrix protein secreted by astrocytes, in promoting central nervous system synaptogenesis (Eroglu et al. 2009). Neuronal $Ca_v\alpha_2\delta_1$ overexpression in transgenic mice results in increased excitatory synapse numbers in the brain. TSP treatment on retinal ganglion cells with $Ca_v\alpha_2\delta_1$ overexpression results in a 100 % increase in the number of synapses, which can be blocked by the $Ca_v\alpha_2\delta_1$ ligand gabapentin. L-, N- or P/Q-type VGCC blockers fail to inhibit TSP-induced synapse formation, suggesting that the roles of $Ca_v\alpha_2\delta$ in synapse formation are not likely associated with VGCC functions.

Consistent with this notion, Purkinje cells in *ducky* mice lacking $Ca_v\alpha_2\delta_2$ have abnormal synapse formation (Brodbeck et al. 2002). $Ca_v\alpha_2\delta_3$ null mutant drosophila embryos lack boutons in neuromuscular junctions of $Ca_v\alpha_2\delta_3$ mutant terminals due to missing ankyrin2-XL, a protein stabilizes synapses by anchoring cell surface proteins in synaptic terminals, that disturbs cytoskeleton arrangement (Kurshan et al. 2009). Boutons are restored by re-expressing $Ca_v\alpha_2\delta_3$ in $Ca_v\alpha_2\delta_3$ null embryos, suggesting that $Ca_v\alpha_2\delta_3$ is involved in the formation of nerve terminals. This process is unlikely to depend on VGCC-related actions since pore forming $Ca_v\alpha_1$ mutant embryos have normal ankyrin2 expression and boutons in nerve terminals (Brodbeck et al. 2002).

15.4.4 Implication of $Ca_v\alpha_2\delta$ Dysregulation in Pain Processing

Three types of $Ca_v\alpha_2\delta$ ($Ca_v\alpha_2\delta_1$, $Ca_v\alpha_2\delta_2$ and $Ca_v\alpha_2\delta_3$) mRNA are identified in primary sensory neurons in DRG (Cole et al. 2005). $Ca_v\alpha_2\delta_1$ and $Ca_v\alpha_2\delta_2$ mRNAs are highly expressed in small DRG neurons but with low expression in large DRG neurons, whereas $Ca_v\alpha_2\delta_3$ mRNA is only present in large DRG neurons (Yusaf et al. 2001). These data suggest that $Ca_v\alpha_2\delta$ subunits may play unique roles in sensory information processing.

The involvement of $Ca_v\alpha_2\delta$ in pain processing is further supported by pharmacology data indicating that gabapentinoids, including gabapentin and pregabalin, have high binding affinity for VGCC $Ca_v\alpha_2\delta_1$ and $Ca_v\alpha_2\delta_2$ subunits (Gee et al. 1996; Marais et al. 2001), and anti-nociception properties in animal models (Hwang and Yaksh 1997; Luo et al. 2001, 2002) and patients (Dworkin and Kirkpatrick 2005; Guay 2005; Zareba 2005). Mutations at the gabepentin binding site within the α_2 peptide (R217A) eliminate gabapentin binding and its anti-nociceptive actions (Field et al. 2006), further confirmed that binding of gabapentinoids to $Ca_v\alpha_2\delta$ proteins may underlie the anti-nociceptive actions of these drugs.

Under pathological conditions that lead to the development of behavioral hypersensitivities, such as peripheral nerve injury and diabetic neuropathies, $Ca_v\alpha_2\delta_1$ upregulation has been reported in dorsal root ganglia and dorsal spinal cord of pain models that correlates with the development of thermal and mechanical hypersensitivities (Luo 2000, 2004; Luo et al. 2001, 2002; Newton et al. 2001; Yusaf et al. 2001; Li et al. 2006). Interestingly, $Ca_v\alpha_2\delta_2$ and $Ca_v\alpha_2\delta_3$ mRNA are

Table 15.1 Dysregulation of voltage gated calcium channel $Ca_v\alpha_2\delta$ subunit in pain models

$Ca_v\alpha_2\delta$ subunit	Dysregulation	Model	Behavioral hypersensitivity	References
$Ca_v\alpha_2\delta_1$	↑ in DRG, DSC	SNL SNTx CCI DNP Paclitaxel-evoked neuropathy SCI Partial sciatic nerve injury	Tactile allodynia, mechanical and thermal hyperalgesia	Luo et al. (2001, 2002), Newton et al. (2001), Yusaf et al. (2001), Valder et al. (2003), Li et al. (2004), Xiao et al. (2007), Bauer et al. (2009), Kim et al. (2009), and Boroujerdi et al. (2011)
$Ca_v\alpha_2\delta_2$	↓ in DRG (mRNA)	SNL	Tactile allodynia	Bauer et al. (2009)
$Ca_v\alpha_2\delta_3$	↓ in DRG (mRNA)	SNL	Tactile allodynia	Bauer et al. (2009)
$Ca_v\alpha_2\delta_4$	ND			

SNL spinal nerve ligation, *SNTx* spinal nerve transection, *CCI* chronic constriction injury of the sciatic nerve, *DNP* Diabetic neuropathy, *SCI* spinal cord injury. *ND* not determined

downregulated after peripheral nerve injury, suggesting a dominant role of $Ca_v\alpha_2\delta_1$ over $Ca_v\alpha_2\delta_2$ and $Ca_v\alpha_2\delta_3$ in peripheral nerve injury-induced pain processing (Bauer et al. 2009) (Table 15.1).

This is confirmed by in vivo findings that $Ca_v\alpha_2\delta_1$ upregulation is required for the onset (Boroujerdi et al. 2008) as well as maintenance of neuropathic pain states (Luo et al. 2001); The antihyperalgesic effects of gabapentin are correlated with upregulation of $Ca_v\alpha_2\delta_1$ subunit in neuropathic pain models (Luo et al. 2002); Blocking injury signals that trigger $Ca_v\alpha_2\delta_1$ upregulation or blocking injury-induced $Ca_v\alpha_2\delta_1$ upregulation directly in a nerve injury model prevent the development of neuropathic pain states (Boroujerdi et al. 2008).

15.4.5 Presynaptic Modulation of Sensory Pathways by Abnormal $Ca_v\alpha_2\delta_1$ Expression

How does peripheral nerve injury-induced upregulation of $Ca_v\alpha_2\delta_1$ proteins contribute to neuropathic pain states? It has been shown that injury-induced upregulation of $Ca_v\alpha_2\delta_1$, but not $Ca_v\alpha_2\delta_2$, proteins in DRG are translocated to presynaptic terminals of sensory afferents in dorsal spinal cord (Li et al. 2004; Bauer et al. 2009). Several lines of evidence support that upregulated $Ca_v\alpha_2\delta_1$ at the presynaptic terminals of sensory afferents in dorsal spinal cord plays a critical role in mediating dorsal horn neuron sensitization and pain processing. (1) Only protein, but not mRNA, levels are upregulated in spinal cord suggesting that injury-induced

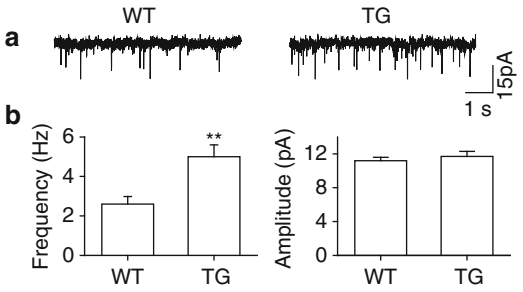

Fig. 15.1 Increased frequency, but not amplitude, of AMPA receptor mediated mEPSCs in dorsal spinal cord neurons of the $Ca_v\alpha_2\delta_1$ transgenic mice. (**a**) Representative traces of mEPSCs from dorsal spinal cord of wild type (WT) and $Ca_v\alpha_2\delta_1$ transgenic (TG) mice, respectively. (**b**) Summary of mEPSC frequency (*left*) and amplitude (*right*) from WT and TG mice, respectively. Data presented are means ± SEM from at least 15 neurons in each group. ** $p < 0.01$ compared with WT neurons by Students' *t* test

$Ca_v\alpha_2\delta_1$ dysregulation mainly occurs at the DRG level, which results in enhanced anterograde axonal transport of the elevated $Ca_v\alpha_2\delta_1$ to the presynaptic terminals of sensory afferents in dorsal spinal cord (Luo et al. 2001; Bauer et al. 2009). (2) Dorsal rhyzotomy that interrupts the anterograde axonal transport of $Ca_v\alpha_2\delta_1$ can block injury-induced $Ca_v\alpha_2\delta_1$ upregulation in dorsal spinal cord and reverse neuropathic pain states (Li et al. 2004). (3) Intrathecal $Ca_v\alpha_2\delta_1$ antisense oligodeoxynucleotide treatment abolishes injury-induced $Ca_v\alpha_2\delta_1$ upregulation in dorsal spinal cord, not in DRG, which correlates with a reversal of neuropathic pain states (Li et al. 2004). (4) Intrathecal injections with glutamate receptor antagonists eliminate behavioral hypersensitivity in spinal nerve ligated rats with $Ca_v\alpha_2\delta_1$ upregulation in DRG and dorsal spinal cord, and $Ca_v\alpha_2\delta_1$-overexpressing mice (Chaplan et al. 1997; Nguyen et al. 2009), suggesting that $Ca_v\alpha_2\delta_1$ mediates behavioral hypersensitivity by facilitating glutamate release at the spinal level. (5) Biochemical data indicate that $Ca_v\alpha_2\delta_1$ can regulate the evoked release of neurotransmitters, such as glutamate, GABA, Substance P, by enhancing the function of presynaptic VGCC, which is sensitive to blockade by gabapentinoids (Quintero et al. 2011). (6) Electrophysiological data indicate that the frequency, but not amplitude, of glutamate (AMPA) receptor-mediated miniature excitatory postsynaptic currents (mEPSC) is increased in $Ca_v\alpha_2\delta_1$-overexpressing transgenic mice (Nguyen et al. 2009) (Fig. 15.1). Since increased frequency of AMPA-receptor mediated mEPSC is a reflection of increased presynaptic release of glutamate, this suggests that elevated $Ca_v\alpha_2\delta_1$ promotes presynaptic glutamate release at the spinal cord level that, in turn, causes dorsal horn neuron sensitization, and behavioral hypersensitivity.

Using immunostaining techniques, Bauer el al. have reported that spinal nerve ligation injury leads to increased $Ca_v\alpha_2\delta_1$ immunoreactivity in axons of the fasciculus gracilis ascending from injured DRG rostrally up to the brainstem (Bauer et al. 2009). Chronic pregabalin treatment in the spinal nerve injured animals reduces this axonal increase of $Ca_v\alpha_2\delta_1$ immunoreactivity when compared with saline control

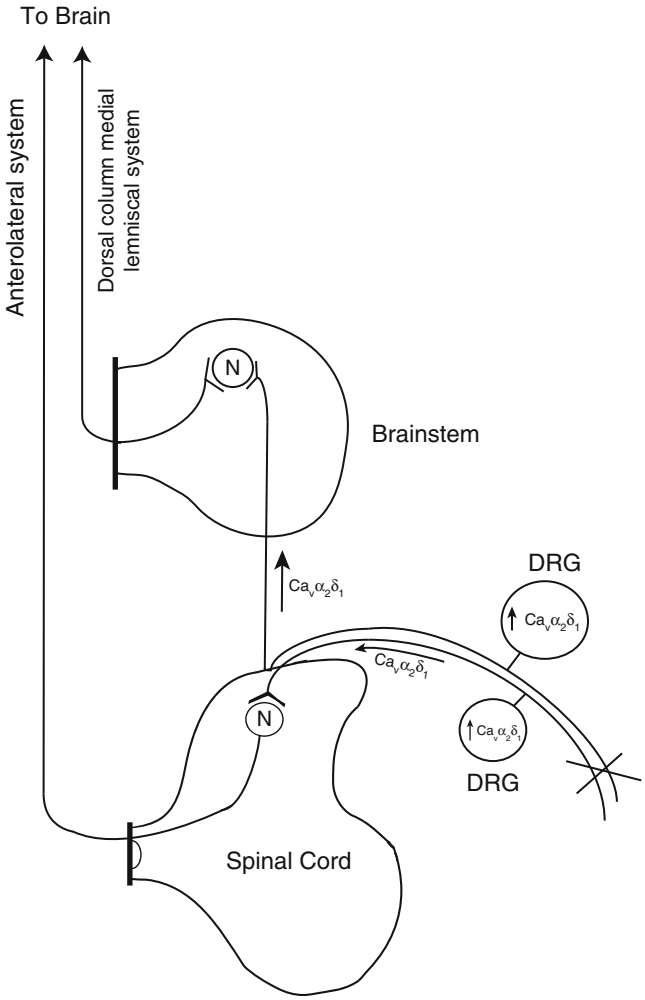

Fig. 15.2 Possible influence of elevated $Ca_v\alpha_2\delta_1$ at different locations along the sensory pathway. Schematic illustration showing how injury induced upregulation of $Ca_v\alpha_2\delta_1$ in DRG could be translocated to multiple locations along the sensory pathway, thus affect presynaptic neurotransmission at these sites. *N* neuron, *X* nerve injury

treatment, suggesting that injury-induced DRG $Ca_v\alpha_2\delta_1$ expression could reach presynaptic terminals of sensory afferents at the lower brainstem level to regulate local presynaptic neurotransmission. This change could affect the excitability of postsynaptic projection neurons sending ascending axons rostrally along the dorsal column medial lemniscal system (Fig. 15.2). In vivo or in vitro electrophysiological recording at that level from peripheral nerve injured animals is warranted to further

test this hypothesis. In vitro studies have suggested that once in the presynaptic terminals, $Ca_v\alpha_2\delta_1$ proteins modulate presynaptic neurotransmission through two possible molecular mechanisms. First, elevated $Ca_v\alpha_2\delta_1$ proteins could increase the membrane expression of presynaptic VGCC. Second, elevated $Ca_v\alpha_2\delta_1$ proteins could increase release probability of neurotransmitter by presumably configuring presynaptic VGCC more favorable for driving exocytosis. The latter requires the presence of the MIDAS motif within the predicted VWA domain of $Ca_v\alpha_2\delta_1$ proteins (Hoppa et al. 2012). Whether similar mechanisms occur in vivo remains to be explored.

Alternatively, $Ca_v\alpha_2\delta_1$ proteins may modulate sensory information processing through activities unrelated to VGCC. Recently, it has been shown that $Ca_v\alpha_2\delta_1$ proteins are critical in promoting excitatory synaptogenesis by serving as neuronal receptors for TSP (Eroglu et al. 2009; Kurshan et al. 2009). VWA domain within $Ca_v\alpha_2\delta_1$ is critical for its interaction with TSP proteins. Importantly, TSP4 is recently identified as a pro-nociceptive factor, which is overly expressed in activated astrocytes in dorsal spinal cord post peripheral nerve injury that leads to enhancing pre-synaptic neurotransmission, dorsal horn neuron sensitization and neuropathic pain processing (Kim et al. 2012). Together, it is likely that increased $Ca_v\alpha_2\delta_1$ in dorsal spinal cord presynaptic terminals of sensory afferents interacts with TSP4 secreted from activated astrocytes to promote formation of excitatory synapses, which can lead to exaggerated neurotransmitter release upon peripheral stimulation and pain sensations. Further studies are required to reveal this potential mechanism of pain processing.

15.4.6 Descending Modulatory Pathways Regulated by $Ca_v\alpha_2\delta_1$

Descending pain modulatory pathways from the cortex, thalamus and brainstem send both inhibitory and facilitatory inputs to the dorsal horn to modulate sensory input from primary afferents in dorsal spinal cord. The release of serotonin, norepinephrine and endogenous opioids from descending pathways can modulate the release of excitatory neurotransmitters, excitatory and inhibitory interneuron activity as well as projection neuron sensitivity at the spinal level. Impairment of these descending modulation pathways often leads to development of chronic pain states.

$Ca_v\alpha_2\delta$ subunits are also expressed in discrete supraspinal regions along descending modulatory pathways (Cole et al. 2005). It has been shown that intracerebroventricular (i.c.v.) administration of gabapentin and pregabalin can reduce thermal and mechanical hypersensitivities in a pain model of peripheral nerve injury without affecting acute thermal and mechanical nociception. These anti-hyperalgesic effects of gabapentinoids correlate with the accelerated spinal turnover of noradrenaline. Following noradrenaline depletion by intracisternal

injection of 6-hydroxydopamine, i.c.v. administration of pregabalin has no effect on thermal and mechanical hypersensitivities. These findings support that gabapentinoids activate the descending noradrenergic pain inhibitory pathway supraspinally in alleviating pain states post nerve injury (Tanabe et al. 2005; Takeuchi et al. 2007a, b).

Similarly, Hayashida et al. have injected gabapentin directly into locus coeruleus (LC) in the pons, and reported that gabapentin reduces behavioral hypersensitivity in spinal nerve ligated rats in a dose-dependent manner, which can be blocked by intra-LC injection of idazoxan, an α2-adrenoceptor antagonist (Hayashida et al. 2008). In addition, data from an in vitro patch clamp recording in LC slices have shown that bath application of gabapentin dose-dependently inhibits $GABA_A$ receptor-mediated, evoked inhibitory postsynaptic currents (IPSC) with increased paired-pulse ratio from peripheral nerve injury mice, but has no effect on IPSC from sham control mice. In contrast, gabapentin treatments do not affect glutamate-mediated evoked excitatory postsynaptic currents (EPSC) in LC of nerve injury mice (Takasu et al. 2008). The authors concluded that gabapentin inhibits GABAergic synaptic transmission in LC through a presynaptic mechanism and subsequently removes inhibitory effects on LC neurons and activates descending noradrenergic inhibition under a neuropathic pain inducing condition (nerve injury). Together, these findings suggest that gabapentin acts directly or indirectly on noradrenergic neurons in the brainstem to stimulate descending inhibition after peripheral nerve injury. This is supported by a clinical study in human indicating that oral gabapentin before surgery significantly increases norepinephrine concentration in cerebrospinal fluid (Hayashida et al. 2007). Because $Ca_v\alpha_2\delta_1$ subunit is the only known receptor for gabapentin and pregabalin, and is dysregulated after peripheral nerve injury, it is possible that gabapentin and pregabalin modulate a noradrenergic descending pathway through binding to the $Ca_v\alpha_2\delta_1$ subunit at the supraspinal level.

Recent studies also suggest that activation of descending 5-HT_3 facilitatory pathway is required for the processing of nociceptive signals in normal and nerve injured animals, as well as the state-dependent inhibitory actions of pregabalin in late stages of nerve injury in a neuropathic pain model (Bee and Dickenson 2008). Ablation of descending facilitatory cells expressing the mu-opioid receptor in rostral ventromedial medulla renders pregabalin ineffective in inhibiting spinal neuron activity, which can be restored by intrathecal injection of a $5HT_3$ receptor agonist to mimic the descending drive at the spinal level (Bee and Dickenson 2008). This suggests that injury-induced $Ca_v\alpha_2\delta_1$ dysregulation, which usually occur in a late stage of nerve injury (Li et al. 2004), may mediate neuropathic pain states through a 5-HT_3 receptor-dependent pathway. To test this hypothesis, we have examined if the descending 5-HT_3 facilitatory pathway is involved in mediating pain states induced by $Ca_v\alpha_2\delta_1$ upregulation at the spinal level by comparing the effects of a 5-HT_3 receptor antagonist in behavioral hypersensitivities in the neuropathic pain model of spinal nerve ligation and $Ca_v\alpha_2\delta_1$ overexpressing transgenic mice. Our findings have indicated that intrathecally, but not systematically, injected ondansetron, a 5-HT_3 receptor antagonist, can block dose-dependently mechanical and thermal

hypersensitivities in both the nerve injury model and injury-free $Ca_v\alpha_2\delta_1$ overexpressing transgenic mice (Chang et al. 2012). Together, these findings support that the serotonergic descending facilitation pathway is involved in central sensitization and pain states mediated by $Ca_v\alpha_2\delta_1$ upregulation, either induced by peripheral nerve injury or transgenic $Ca_v\alpha_2\delta_1$ overexpression, at the spinal level.

15.5 Perspectives

Structure, cellular/molecular biology, and pathophysiological functions of $Ca_v\alpha_2\delta$ subunits have been extensively studied in the last two decades. Moreover, a large body of emerging evidence indicates that $Ca_v\alpha_2\delta$ subunit is a multifunctional protein. It regulates not only pathophysiological functions of VGCC, but also VGCC-independent functions. The following important questions regarding the functions of $Ca_v\alpha_2\delta$ subunits in disease states remain to be elucidated.

1. What is the functional implication of $Ca_v\alpha_2\delta$ dysregulation in modulation of VGCC trafficking and functions, facilitation of synaptic neurotransmission, and alterations in neural circuits in disease states?
2. In addition to TSP and ankyrin2-XL, which other proteins interact with $Ca_v\alpha_2\delta$ under different pathological conditions? What are the signaling pathways underlying $Ca_v\alpha_2\delta$ mediated pathological conditions such as pain processing?
3. Is $Ca_v\alpha_2\delta$ dysregulation in sensory neurons cell-type specific? If so, what is the implication of cell-type specific $Ca_v\alpha_2\delta$ dysregulation and its neuraxial distribution in mediating modality specific behavioural hypersensitivity?
4. What are the factors and signalling pathways involved in mediating $Ca_v\alpha_2\delta$ dysregulation under pathological conditions?

Discoveries leading to the understanding of these questions will provide us with a new insight into disorders related to $Ca_v\alpha_2\delta$ dysregulation and lead to the development of new and target specific medications for management of disorders involving $Ca_v\alpha_2\delta$ dysregulation.

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