The Anti-Allodynic Gabapentinoids: Myths, Paradoxes and Acute Effects

Article *in* The Neuroscientist · April 2016

Impact Factor: 6.84 · DOI: 10.1177/1073858416628793

2 authors:

Sascha R Alles

University of British Columbia - Vancouver

6 PUBLICATIONS **1** CITATION

SEE PROFILE

Peter A Smith

University of Alberta

143 PUBLICATIONS **2,508** CITATIONS

SEE PROFILE

The Neuroscientist
1–16

DOI: 10.1177/1073858416628793
nro.sagepub.com
SAGE

The Anti-Allodynic Gabapentinoids: Myths, Paradoxes, and Acute Effects

Sascha R. A. Alles[1] and Peter A. Smith[1]

Abstract

The gabapentinoids (pregabalin and gabapentin) are first line treatments for neuropathic pain. They exert their actions by binding to the α2δ accessory subunits of voltage-gated Ca^{2+} channels. Because these subunits interact with critical aspects of the neurotransmitter release process, gabapentinoid binding prevents transmission in nociceptive pathways. Gabapentinoids also reduce plasma membrane expression of voltage-gated Ca^{2+} channels but this may have little direct bearing on their therapeutic actions. In animal models of neuropathic pain, gabapentinoids exert an anti-allodynic action within 30 minutes but most of their in vitro effects are 30-fold slower, taking at least 17 hours to develop. This difference may relate to increased levels of α2δ expression in the injured nervous system. Thus, in situations where α2δ is experimentally upregulated in vitro, gabapentinoids act within minutes to interrupt trafficking of α2δ subunits to the plasma membrane within nerve terminals. When α2δ is not up-regulated, gabapentinoids act slowly to interrupt trafficking of α2δ protein from cell bodies to nerve terminals. This improved understanding of the mechanism of gabapentinoid action is related to their slowly developing actions in neuropathic pain patients, to the concept that different processes underlie the onset and maintenance of neuropathic pain and to the use of gabapentinoids in management of postsurgical pain.

Keywords

neuropathic pain, alpha-2-delta ligand, calcium channels, neurotransmitter release, time course

Introduction

By signaling actual or potential tissue damage, pain protects from injury and enables survival and procreation. By contrast, injury to the somatosensory system can produce maladaptive "neuropathic" pain that lasts for months or years after any injury has healed (Costigan and others 2009; Moulin and others 2014). This "disease of pain" has a 1.5% to 3% prevalence within the general population (Gilron and others 2006; Taylor 2006; Torrance and others 2006; Torrance and others 2013), suggesting that as many as 210,000,000 people are afflicted worldwide. Neuropathic pain can be associated with diabetic, postherpetic, or HIV-related neuropathies, with multiple sclerosis or fibromyalgia as well as with traumatic nerve, spinal cord or brain injury (including stroke). It is characterized by touch-induced pain (allodynia), heightened responses to noxious stimuli (hyperalgesia) and may be associated with an ongoing "burning" pain (causalgia) and "electric shock-like" bouts of spontaneous pain that are independent of sensory activation.

Neuropathic pain is poorly responsive to traditional analgesics such as non-steroidal anti-inflammatory drugs (NSAIDs) and to opioids, and this can lead to the overprescription and abuse. First line treatment thus includes the anti-allodynic gabapentinoids, pregabalin, and gabapentin (Finnerup and others 2015; Martinotti and others 2013; Moulin and others 2007; Moulin and others 2014). Here we will review the current understanding of gabapentinoid effectiveness in neuropathic pain. In so doing, we hope to dispel some misconceptions relating to their mechanism of action and to resolve two paradoxes relating to their time course of effect.

Sensory Processing in Neuropathic Pain

Many laboratory studies of neuropathic pain focus primarily on the consequences of chronic peripheral nerve

[1]Neuroscience and Mental Health Institute and Department of Pharmacology, University of Alberta, Edmonton, Alberta, Canada

Corresponding Author:
Peter A. Smith, Department of Pharmacology and Neurosciences and Mental Health Institute, Faculty of Medicine and Dentistry, University of Alberta, 9-75 Medical Sciences Building, Edmonton, Alberta, T6G 2H7, Canada.
Email: pas3@ualberta.ca

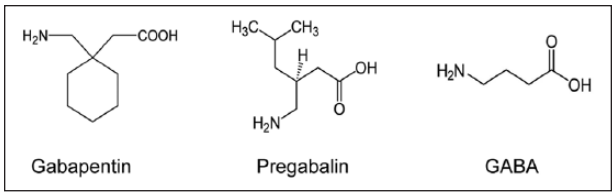

Figure 1. Structure of GABA and the gabapentinoid drugs for neuropathic pain.

injury in rodents (Decosterd and Woolf 2000; Kim and others 1997). This leads to mechanical allodynia, which is initiated by ectopic discharges in primary afferent fibers (Dib-Hajj and others 2010; Pitcher and Henry 2008; Sandkuhler 2009; von Hehn and others 2012; Wall and Devor 1983; Waxman and Zamponi 2014) and "central sensitization" wherein neurons in the nociceptive circuitry of the spinal dorsal horn become susceptible to activation by innocuous peripheral stimuli (Baranauskas and Nistri 1998; Berger and others 2011; Dalal and others 1999; Sandkuhler 2009; Woolf 1983). Central sensitization has been described as a "pathological learning process" (Woolf 1983) and several articles explore the relationship between central sensitization and classical neuronal learning processes (Fenselau and others 2011; Gruber-Schoffnegger and others 2013; Ruscheweyh and others 2011; Sandkuhler and Gruber-Schoffnegger 2012). Aberrations in sensory processing at the level of the spinal dorsal horn are marked by increases in the release of excitatory neurotransmitters, an increase in excitatory synaptic drive, a decrease in inhibitory synaptic drive as well as suppression of GABA and/or glycine-mediated post synaptic inhibition (Balasubramanyan and others 2006; Chen and others 2009; Coull and others 2003; Coull and others 2005; Lee and Prescott 2015; Leitner and others 2013; Lu and others 2009; Sandkuhler 2009). Many of these effects are driven by release of brain-derived neurotrophic factor (BDNF) from activated microglia (Biggs and others 2010; Coull and others 2005; Lu and others 2009) as well as injury-induced T-cell infiltration (von Hehn and others 2012) and the actions of pro-inflammatory cytokines and chemokines (Grace and others 2014).

Neuropathic pain is also associated with changes in thalamic, limbic, autonomic, and cortical structures, including changes in the size of areas involved in sensory and affective processing and changes in the release of excitatory and inhibitory neurotransmitters (Gustin and others 2012; Lin and others 2014; Masocha 2015; Xu and others 2008; Zhuo 2008). This so-called "pain matrix" includes the medial prefrontal cortex, nucleus accumbens, anterior cingulate cortex, insula, amygdala, periaqueductal gray, locus coerulus, and rostrovental medulla (von Hehn and others 2012). It is also generally accepted that processes responsible for the initiation of neuropathic pain may differ from those responsible for its long-term maintenance. Microglia activation may be primarily associated with pain onset and astrocytes may contribute to the persistence of pain over periods of months and years (Grace and others 2014; Zhang and de Koninck 2006).

Therapies for neuropathic pain attempt to counteract the resultant enduring changes in neuronal excitability. Partial success may be achieved using the gabapentinoids (pregabalin and gabapentin) and/or serotonin/noradrenaline uptake inhibitors and/or topical capsaicin application (Finnerup and others 2015; Moulin and others 2014; Sindrup and Jensen, 1999). Unfortunately, these first line treatments are far from universally effective. Gabapentinoids bring relief in only 35% of patients (Moore and others 2014).

The Gabapentinoids

Gabapentin was designed as a GABA mimetic with increased lipophilicity so as to improve access to the central nervous system. Since many forms of epilepsy involve dysfunctional GABAergic transmission, gabapentin was first studied as an anticonvulsant (Kondo and others 1991; Sivenius and others 1991). It was approved by the Food and Drug Administration in 1993 as an adjunct therapy for partial seizures in patients older than 12 years. However, it was later approved for children aged 3 to 12 years in 2000 for the same indication and in 2004, gabapentin was approved for the treatment of postherpetic neuralgia in adults (Mack 2003). Since gabapentin came on to the market, there has been widespread off-label use for bipolar disorder, attention deficit/hyperactivity disorder, restless leg syndrome, drug and alcohol withdrawal seizures, and sleep disorders (Mack 2003). It has also been shown that the paradoxical effect of long-term opioid administration, which can lead to opioid-induced hyperalgesia, can be mitigated by gabapentin treatment (Stoicea and others 2015).

A recent meta-analysis concluded that gabapentin produces meaningful pain reduction of at least 50% compared with placebo in cases of post-herpetic neuralgia and painful diabetic neuropathy; however, information regarding usefulness in other pain conditions is inconclusive (Moore and others 2014). It was also found that 66% of patients taking gabapentin experienced an adverse event, which included dizziness or drowsiness and less commonly, gait disturbance or peripheral edema (Moore and others 2014).

A second gabapentinoid, pregabalin (*S*-(+)-3-isobutyl GABA) was also developed as a GABA mimetic as a successor to gabapentin (Fig. 1) (McClelland and others 2004; Tzellos and others 2010). It was approved in 2004

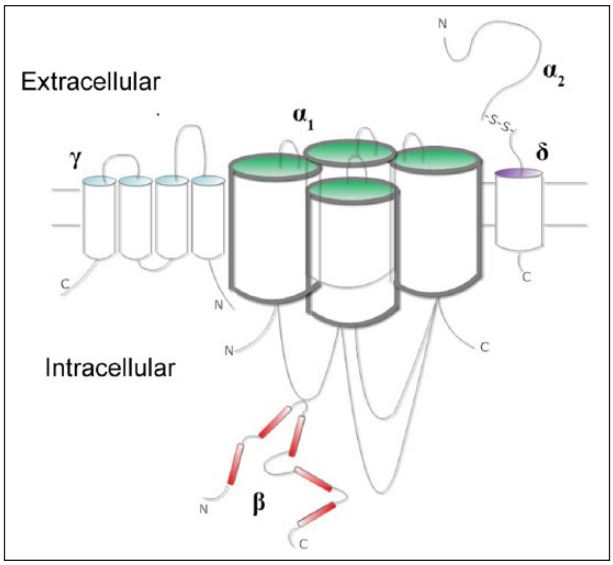

Figure 2. The voltage-gated calcium channel. Diagram to illustrate the structure of high-voltage activated (HVA) Ca^{2+} channels showing interactions of the pore-forming α_1 subunit and the auxiliary $\alpha_2\delta$, β, and γ subunits. The α_2 accessory subunit is entirely extracellular and is linked via a disulfide bridge to the δ subunit, which is mainly extracellular with a short transmembrane and intracellular domain. The β subunit is entirely intracellular.

for treatment of epilepsy and neuropathic pain syndromes, namely painful diabetic neuropathy (Dworkin and Kirkpatrick 2005). In addition, pregabalin was approved for the treatment of anxiety disorders in Europe (Tzellos and others 2010).

Despite their structural similarity to GABA (Fig. 1) neither pregabalin nor gabapentin bind strongly to $GABA_A$ or $GABA_B$ receptors (Lanneau and others 2001; Li and others 2011; Moore and others 2002; Sutton and others 2002). Gabapentinoids also do not affect GABA uptake, synthesis, or metabolism (Taylor and others 2007). The first insight into their mechanism of action came 20 years ago when Gee and others (1996) isolated and sequenced a protein that bound gabapentin from porcine brain and identified it as the α2δ-1 subunit of voltage-gated calcium channels or $Ca_V\alpha2\delta1$. Gabapentinoids are thus referred to as α2δ ligands (Dooley and others 2007). The physiological role of α2δ subunits and their likely involvement in the etiology of neuropathic pain is covered in the next few paragraphs.

Voltage-Gated Calcium Channels and Their α2δ Accessory Subunits

A detailed description of voltage-gated calcium channels is beyond the scope of this review, but may be found in two recent reviews (Simms and Zamponi 2014; Zamponi 2016). Briefly, these channels encompass high-voltage activated (HVA) L-types ($Ca_V1.1$, $Ca_V1.2$, $Ca_V1.3$, and $Ca_V1.4$); P/Q-type ($Ca_V2.1$,) N-type ($Ca_V2.2$), and R-type ($Ca_V2.3$) as well as T-type (low-voltage-activated, LVA) Ca^{2+} channels ($Ca_V3.1$, $Ca_V3.2$, $Ca_V3.3$) (Zamponi, 2015). Influx of Ca^{2+} via HVA Ca^{2+} channels triggers neurotransmitter release from presynaptic vesicles and thereby determines neuronal network excitability. The importance of HVA-Ca^{2+} channels in neuropathic pain is illustrated by the clinical effectiveness of the N-type Ca^{2+} channel blocker ziconotide (Zamponi and others 2015) and as will be discussed below, the relationship between HVA-Ca^{2+} channel function and the actions of gabapentinoids.

Voltage-gated Ca^{2+} channels consist of five subunits: the α_1 pore-forming subunit and auxiliary subunits α2, β, δ, and γ (Fig. 2; reviewed in Zamponi and others 2015). The main subtype found in presynaptic terminals is Ca_V2 (Westenbroek and others 1992). $Ca_V2.1$ and $Ca_V2.2$ both contain a synaptic protein interaction site (synprint) that interacts with SNARE proteins (syntaxin and SNAP-25) (Rettig and others 1996; Sheng and others 1994). By this mechanism, channels can be closely associated with synaptic vesicles that govern release of neurotransmitter.

The α2δ subunits, which bind and mediate the effects of gabapentinoids (Bauer and others 2010a; Field and others 2006), are multifunctional and are expressed in the plasma membrane in multimeric complexes with mature HVA-Ca^{2+} channels. T-type (LVA) Ca^{2+} channels ($Ca_V3.1$, $Ca_V3.2$, $Ca_V3.3$) do not appear to associate directly with α2δ proteins (Dolphin 2013; Lacinova and others 2000).

Four different mammalian genes encode the α2δ subunits: *CACNA2D1-CACNA2D4* (Whittaker and Hynes 2002). Of the four available types (α2δ-1 through α2δ-4) (Dolphin, 2012), the α2δ-1 subunit is expressed in primary afferent nerve fibers and is crucial for release of excitatory neurotransmitter from their terminals in the spinal dorsal horn (Hoppa and others 2012).

α2δ subunits are glycophosphatidylinositol (GPI)-anchored (Fig. 2; Davies and others 2010). α2δ-1 subunits have been shown to increase Ca_V2 plasma membrane expression suggesting that part of the role of α2δ is in trafficking of channel complexes (Cassidy and others 2014). While T-type (Ca_V3) channels do not require α2δ to be expressed, their expression is enhanced by the presence of α2δ (Zamponi and others 2015).

α2δ Subunits and Neuropathic Pain

Deletion of the α2δ-1 gene in animal models delays mechanical hypersensitivity in response to peripheral nerve damage and impedes functional expression of pore forming $Ca_V2.2$ α-subunits in the plasma membrane of the cell bodies of dorsal root ganglion (DRG) neurons

(Patel and others 2013). By contrast, transgenic mice engineered to overexpress α2δ-1 display increased HVA-Ca^{2+} channel current (I_{Ca}) in DRG neurons as well as pain behaviours and prolonged dorsal horn neuronal responses to mechanical and thermal stimulation in the periphery (Li and others 2006). It has also been shown that injury-induced discharges that contribute to the initiation of neuropathic pain are involved in the up-regulation of α2δ-1 levels in the spinal dorsal horn (Boroujerdi and others 2008).

Increased expression of α2δ-1 following nerve injury has thus been strongly implicated in the etiology of neuropathic pain (Boroujerdi and others 2011; Li and others 2006; Zhou and Luo 2015) and binding of gabapentinoids to this subunit likely plays a major role in their anti-allodynic actions (Bauer and others 2009; Boroujerdi and others 2011; Hendrich and others 2008; Luo and others 2002; Zamponi and others 2015).

Mechanism of Gabapentinoid Action

Gabapentinoids are transported into the neuronal cytoplasm via a neutral amino acid transporter (Cheng and Chiou 2006; Su and others 1995) where they bind to α2δ-1 (Bauer and others 2009; Field and others 2006). Interruption of the interaction of α2δ-1 with pore-forming α-subunits of HVA Ca^{2+} channels reduces their trafficking and the appearance of functional channels at the cell surface. This likely involves impediment of the action of a positive regulator of trafficking such as isoleucine (Hendrich and others 2008; Zamponi and others 2015). This has led to the assumption that gabapentinoids also interrupt trafficking of pore forming α-subunits over a longer distance as they are gradually transported from cell bodies of sensory neurons to primary afferent terminals. The resulting decrease in channel availability would be expected to decrease depolarization-induced Ca^{2+} influx and this has been suggested to reduce neurotransmitter release (Bauer and others 2010a; Cheng and Chiou 2006; Field and others 2006; Fink and others 2000; Yang and others 2014). As will be outlined below, this assumption seems to be invalid (Biggs and others 2014; Hoppa and others 2012).

Myth: Gabapentinoids Reduce Neurotransmitter Release by Decreasing Expression of HVA Ca^{2+} Channels on Nerve Terminals

Drug or neurotransmitter modulation of HVA-Ca^{2+} channels on the cell bodies of DRG neurons has for many years been used as a model to predict their action at primary afferent terminals within the dorsal horn (Dunlap and Fischbach, 1981). This concept is illustrated in Figure 3a. The assumption has been made that any drug that reduces HVA-I_{Ca} in DRG cell bodies will exert the same effect on Ca^{2+} channels at nerve terminals and that this will be reflected as reduction in neurotransmitter release. This is not the case for gabapentinoids (Biggs and others 2014). Incubation of cultured DRG neurons for 3 to 4 days with 10 μM pregabalin reduces HVA-I_{Ca} in the cell bodies of small, putative nociceptive, "IB4 negative" neurons to 67% of their control amplitude. This is illustrated in the current-voltage relationship for HVA-Ca^{2+} channels (Fig. 3b). By contrast, acute application of a low concentration of Mn^{2+} is considerably more effective; 200 μM Mn^{2+} reduces HVA-I_{Ca} to 8% of its control amplitude (Fig. 3c). A typical recording of HVA Ca^{2+} current illustrating the strong effect of 200 μM Mn^{2+} is illustrated in Figure 3d. In the dorsal horn, however, 200 μM Mn^{2+} failed to affect synaptic activity in substantia gelatinosa neurons as monitored by the amplitude of spontaneous excitatory postsynaptic currents (sEPSCs; Fig. 3a, e, and g). Despite its relatively small effect on HVA-I_{Ca} in DRG cell bodies, 5- to 6-day exposure of substantia gelatinosa neurons in organotypic culture to 10 μM pregabalin has a clear depressant effect on synaptic transmission as demonstrated by a significant reduction in the amplitude of sEPSCs (Fig. 3f). The summarized findings presented in Figure 3g show that 200 μM Mn^{2+} is more effective in blocking HVA-Ca^{2+} channels than 10 μM pregabalin whereas 10 μM pregabalin is more effective than 200 μM Mn^{2+} in blocking neurotransmitter release. The moderate effect of pregabalin on HVA-I_{Ca} in DRG cell bodies (Fig. 3b) is therefore insufficient to explain its ability to reduce transmitter release in the spinal dorsal horn (Fig. 3f)

These findings may be explained in terms of the results of Hoppa and others (2012) that gabapentinoid inhibition of neurotransmitter release reflects interruption of the ability of α2δ to facilitate interaction of HVA-Ca^{2+} channels with neurotransmitter release sites. Additional evidence for a direct action on the release process is provided by the recent observation that gabapentin reduces the frequency of miniature EPSCs (mEPSCs) in the dorsal horn (Zhou and Luo 2014, 2015). Since mEPSCs are recorded in the presence of tetrodotoxin, they reflect transmitter release that is independent of depolarization and hence the entry of Ca^{2+}-via HVA-Ca^{2+} channels. Thus gabapentin exerts its effect by a mechanism that is distinct from reduced expression of HVA-Ca^{2+} channels in plasma membrane of nerve terminals.

The modest effect of Mn^{2+} on transmitter release may be explained by the classical third- to fourth-power relationship between Ca^{2+} influx and release (Dodge and Rahamimoff 1967); even though the amount of Ca^{2+} entering terminals is reduced in the presence of Mn^{2+}, this is sufficient to support substantial neurotransmitter release. This possibility is supported by the observation

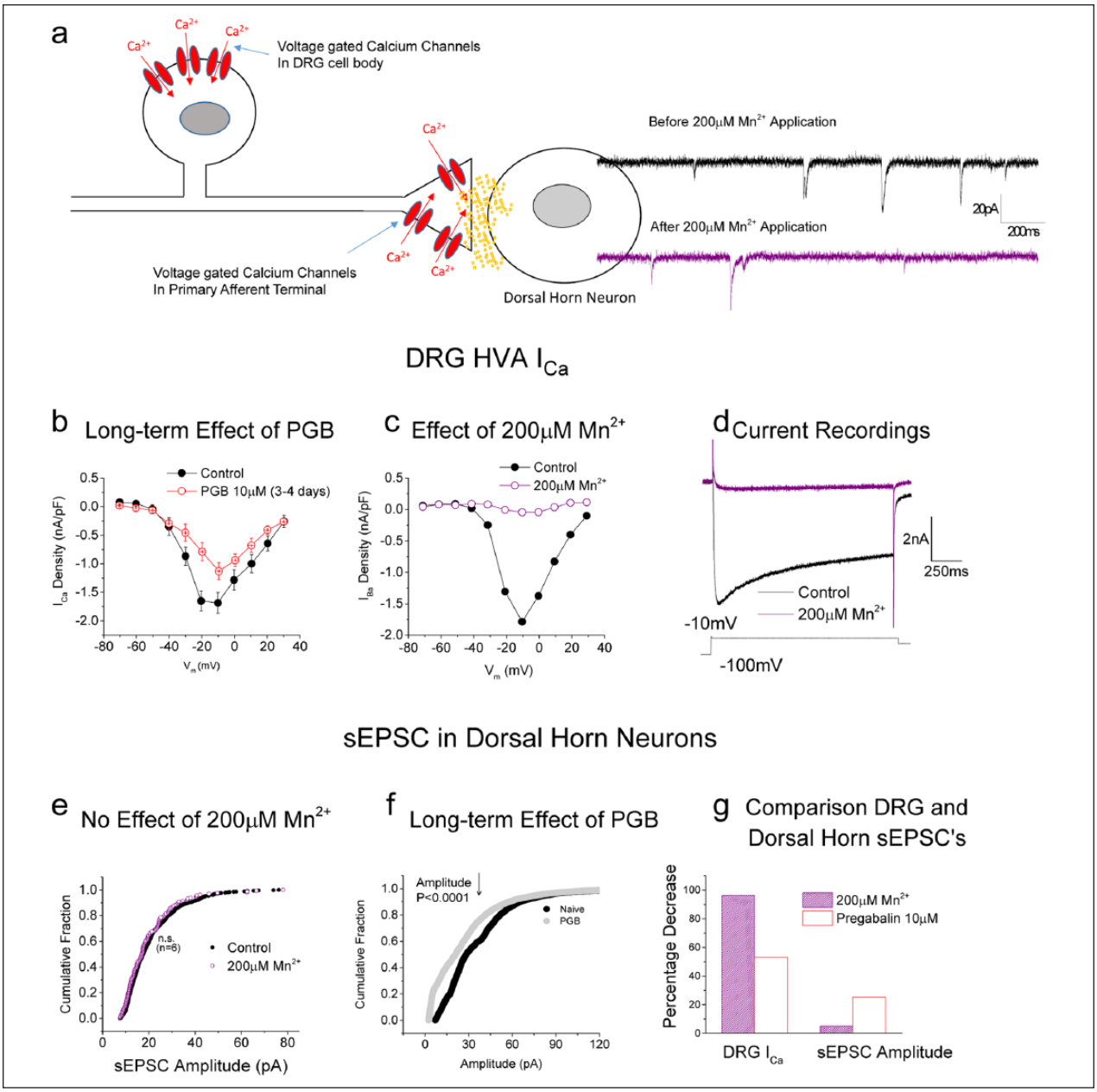

Figure 3. Differential effects of Mn^{2+} and pregabalin (PGB) on HVA-I_{Ca} and nerve terminal activity. (a) Diagram to illustrate the concept that study of voltage gated Ca^{2+} channels in dorsal root ganglion (DRG) neuronal cell bodies may be used as a model for inaccessible Ca^{2+} channels on primary afferent terminals. Release of glutamate (yellow circles) from primary afferent terminals generates excitatory postsynaptic currents (EPSCs) in dorsal horn neurons. Data records at right show spontaneous EPSCs (sEPSC) recorded in a *substantia gelatinosa* neuron in the dorsal horn of a rat spinal cord in the absence and presence of 200 μM Mn^{2+}. (b) Effect of 3- to 4-day exposure to 10 μM pregabalin on HVA-I_{Ca} densit–voltage relationship of small IB4 negative DRG neurons (Ba^{2+} was used as charge carrier). (c) Effect of 200 μM Mn^{2+} on current density–voltage relationship of HVA-I_{Ca} in a small DRG neuron. Note that current is reduced to <10% of its control value. (d) Recording of HVA-I_{Ba} at −10 mV from a small DRG neuron prior to and after the addition of 200μM Mn^{2+}. V_h = −100 mV, voltage command shown in lower trace. Note strong suppression of current by 200 μM Mn^{2+}. (e) Superimposed cumulative probability plots to show lack of effect of 200 μM Mn^{2+} on amplitude of sEPSCs in neurons from *substantia gelatinosa* region of a spinal cord. Data pooled from 5 neurons. (f) Cumulative probability plots of sEPSC amplitude from high threshold, putative excitatory *substantia gelatinosa* neurons in organotypic culture replotted (with permission) from data of Biggs and others (2014). Comparison of control neurons with those exposed to 10 μM pregabalin for 5 to 6 days ($P < 0.0001$, Kolgomorov-Smirnov test). (g) Replotting of data from (b) and (c) and from Biggs and others (2014) as percentage changes to show that 200 μM Mn^{2+} is far more effective than 10 μM pregabalin in attenuating HVA-I_{Ca} in DRG cell bodies. Ten micromolar solution of pregabalin attenuates sEPSC amplitude in *substantia gelatinosa* yet 200 μM Mn^{2+} is almost without effect.

Table 1. Summary of Rapid In Vivo and Slow In Vitro Actions of Gabapentinoids in Animal Models of Neuropathic Pain.

Rapid Effects Occurring in 30-60 Minutes In Vivo	Slow Effects Taking More Than 10 Hours In Vitro
Patel and others (2001): Gabapentin (100 mg/kg) increases paw withdrawal threshold in an inflammatory pain model within 1 hour of IP injection	Heblich and others (2008): 1 mM gabapentin inhibited current through Ca^{2+} channels expressed in TsA201 cells within 17 to 20 hours but not within 3 to 6 hours
Fox and others (2003): Gabapentin produced significant dose-related reversal of tactile allodynia in the rat following a single administration	Hendrich and others (2008): 1 mM gabapentin inhibited current through Ca^{2+} channels expressed in TsA201 cells or native currents in rat DRG neurons within 40 hours but not acutely
Coderre and others (2005): Gabapentin (300 mg/kg) reduces neuropathic pain by inhibiting the spinal release of glutamate	Hendrich and others (2012): 40- to 48-hour exposure to pregabalin (100 µM) inhibits synaptic transmission between rat dorsal root ganglion and dorsal horn neurons in culture
Field and others (2006): Pregabalin (30 or 100 mg/kg) or gabapentin (100 or 300 mg/kg) increases paw withdrawal threshold in a neuropathic pain model within 1 hour of IP injection	Biggs and others (2014): Exposure to 10 µM pregabalin for 5-6 days reduced maximal HVA I_{Ba} density in small IB4-positive DRG neurons
Kumar and others (2013): Attenuation of facial hypersensitivity and noxious stimulus-evoked release of glutamate in medullary dorsal horn in a rodent model of trigeminal neuropathic pain within 30 minutes of IP injection of 1 or 25 mg/kg pregabalin	Biggs and others (2014, 2015): Studies on rat spinal cord in organotypic culture, decreased excitability and excitatory synaptic transmission following 5- to 6-day exposure to 10 µM pregabalin or 100 µM gabapentin
This review, Fig 4a, increase in paw withdrawal threshold in rats subject to CCI following IP injection of 100 mg/kg gabapentin	

CCI = chronic constriction injury; DRG = dorsal root ganglion; HVA = high-voltage activated; IP = intraperitoneally.

that overexpression of pore-forming $Ca_v2.2$ channels in hippocampal neurons fails to increase EPSC size and the suggestion that the strength of neurotransmission is saturated with regard to levels of Ca^{2+} channel expression (Cao and Tsien, 2010).

Do Gabapentinoids Impede Trafficking of Pore Forming Alpha Subunits from Cell Bodies to Nerve Terminals?

The actions of gabapentinoids on DRG or dorsal horn neurons take at least 17 hours to develop in vitro (Biggs and others 2014; Heblich and others 2008; Hendrich and others 2008; Hendrich and others 2012). This is consistent with the suggestion that α2δ ligands prevent the transport of newly synthesized pore-forming Ca^{2+} channel α-subunits from the cell body of DRG neurons to their terminals in the dorsal horn. This is further supported by the observation that long-term gabapentinoid exposure limits the expression of functional HVA-Ca^{2+} channels in the plasma membrane of cell bodies of DRG neurons (Biggs and others 2014; Hendrich and others 2008).

However, in the light of the previous discussion, decreased expression of functional Ca^{2+} channels at nerve terminals may be of little consequence, as their blockade by Mn^{2+} has surprisingly little effect on neurotransmitter release (Fig. 3e). Also, since α2δ participates directly in the neurotransmitter release process per se (Hoppa and others 2012; Zhou and Luo 2014, 2015), the slowly developing effects of gabapentinoids may reflect inhibition of trafficking of α2δ-1 subunits, as opposed to pore forming α subunits, from cell bodies to terminals. This alone would be expected to reduce neurotransmitter release by impeding interaction of HVA-Ca^{2+} channels with the release process. This possibility is supported by the findings of Bauer and others (2009) who showed in nerve injured animals, where α2δ-1 is up-regulated, its trafficking to primary afferent terminals is prevented by chronic pregabalin treatment.

Slowly developing actions of gabapentinoids in vitro do not correlate with their rapid actions in animal models in vivo where antiallodynic effects can be seen within 30 minutes of intraperitoneal injection (Field and others 2006; Kumar and others 2013; Patel and others 2001). We thus define "rapid" effects as those occurring with 30 to 60 minutes to distinguish them from "slow" effects that take 10 to 20 hours to develop. The paradoxical difference between the rapid in vivo and slow in vitro actions of gabapentinoids is discussed in the next section.

Paradox 1: Time Course of Gabapentinoid Action in Animal Models; In Vitro versus In Vivo

A single intraperitoneal injection of 100 mg/kg gabapentin suppresses mechanical allodynia (Fox and others 2003) and other signs of neuropathic pain in animal

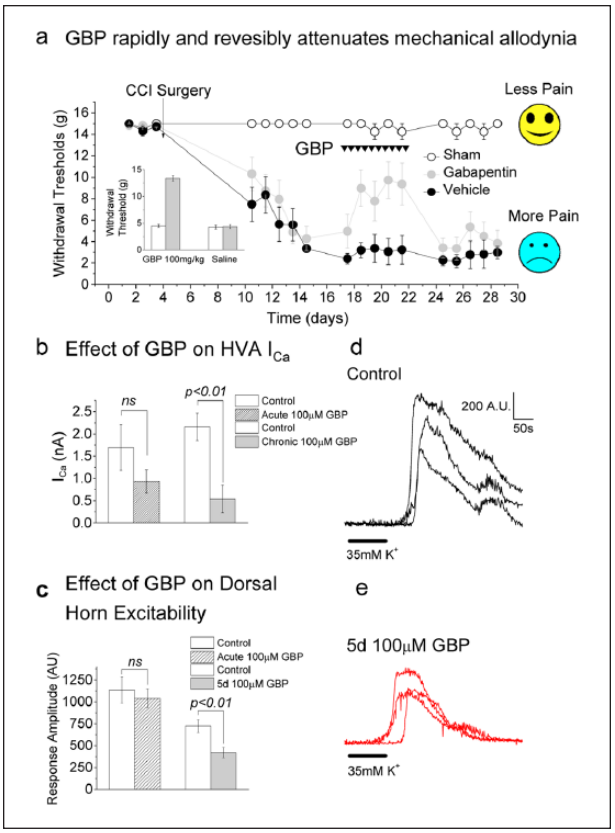

Figure 4. Acute versus chronic effects of gabapentin (GBP) on HVA-I_{Ca} in dorsal root ganglion (DRG) neurons and dorsal horn excitability. (a) Effects of chronic constriction injury (CCI, see Balasubramanyan and others, 2006) on mechanical allodynia measured from withdrawal thresholds to paw stimulation with von Frey filaments. Black downward triangles designate intraperitoneal injections of 100 mg/kg GBP. Note rapid increase in withdrawal threshold indicating suppression of allodynia and return of allodynia within 2 hours of discontinuation of drug injections. Experiments done on four sham-operated animals, 11 animals treated with gabapentin, and 11 animals treated with vehicle (intraperitoneal saline injection). Inset is replot of 30 minute time point data from main graph to further illustrate the rapid onset of gabapentin effect. (b) Comparison of acute and chronic effects of 100 μM GBP on HVA-I_{Ca} in small, putative nociceptive, IB4-negative DRG neurons. Drug was applied to six neurons for 20 minures and no significant effect was seen. By contrast, exposure of cultured DRG neurons to 100 μM GBP for 3 to 4 days produced a significant reduction in current (data from five control neurons and six exposed to drug, $P < 0.01$). (c) Lack of effect of acutely applied GBP (100 μM) on spinal cord excitability as monitored by Ca^{2+} response to 35 mM K^+ challenge ($n = 13$) and significant effect ($P < 0.01$) seen with 5-day GBP exposure. Data from 40 control neurons and 27 exposed to drug. Experiments were done by confocal Ca^{2+} imaging of *substantia gelatinosa* neurons in organotypic culture and were derived from previously published data (Biggs and others, 2014). (d and e) Typical neuronal Ca^{2+} responses to 35 mM K^+ in a control slice or in one treated for 5 days with 100 μM GBP. AU = arbitrary units of Fluo-4 AM (Ca^{2+} indicator) fluorescence.

models within 30 to 60 minutes (Field and others 2006; Kumar and others 2013; Patel and others 2001) yet, as mentioned, most reported actions of gabapentinoids on neurons in vitro are ~30-fold slower, taking 17 hours or more to develop (Biggs and others 2014; Biggs and others 2015; Hendrich and others 2012). For additional detail, see Table 1. Figure 4a shows the reduction of withdrawal threshold for mechanical (von Frey filament) stimulation seen in rats subject to chronic constriction injury (CCI) of their sciatic nerve; lowered mechanical thresholds are indicative of allodynia and hyperalgesia (Kim and others 1997). Intraperitoneal injections of 100 mg/kg gabapentin rapidly and reversibly eliminate these signs and increase mechanical withdrawal threshold toward that seen in uninjured animals.

In line with our observations with pregabalin (Fig. 3b and f) and the consensus that neuronal actions of gabapentinoids in vitro take 17 hours or more to develop (Biggs and others 2014; Biggs and others 2015; Heblich and others 2008; Hendrich and others 2008; Hendrich and others 2012; Zamponi and others 2015), we found that 3- to 4-day exposure to a therapeutically relevant concentration of 100 μM gabapentin (Kushnir and others 1999) significantly reduced HVA I_{Ca} in DRG neurons ($P < 0.01$) whereas acute exposure was without effect (Biggs and others 2014). These findings are summarized in Figure 4d. A similar slowly developing effect of gabapentin was also seen on dorsal horn excitability, but acutely applied drug was also without effect (Fig. 4c). Spinal cord neurons in organotypic slice culture (Biggs and others 2012; Lu and others 2006) were challenged with 35 mM K^+ for 90 seconds and this evoked a large increase in intracellular Ca^{2+} as monitored by confocal Ca^{2+} imaging using Ca^{2+} indicator Fluo-4 AM. This response, which was used as an overall index of dorsal horn excitability, was significantly reduced in slices exposed to gabapentin for 5 to 6 days (Fig. 4c) but was unaffected by acute exposure to 100 μM gabapentin. (Biggs and others 2014). Sample recordings of the effect of 5-day GBP exposure on Fluo-4 fluorescence are shown in Figure 4d and e.

One likely reason for this temporal discrepancy between in vivo and in vitro drug actions is that many in vitro studies have been done on neurons from uninjured, control animals (Biggs and others 2014; Hendrich and others 2012; Moore and others 2002) whereas the rapidly developing behavioral effects are done on nerve injured animals where α2δ-1 is up-regulated (Field and others 2006; Kumar and others 2013;Narita and others 2012). Other work in either neuropathic animals (Coderre and others 2005; Patel and others 2000) or in situations where α2δ-1 is up-regulated (Li and others 2006) have revealed rapidly developing effects of acutely applied gabapentinoids (Zhou and Luo 2015).

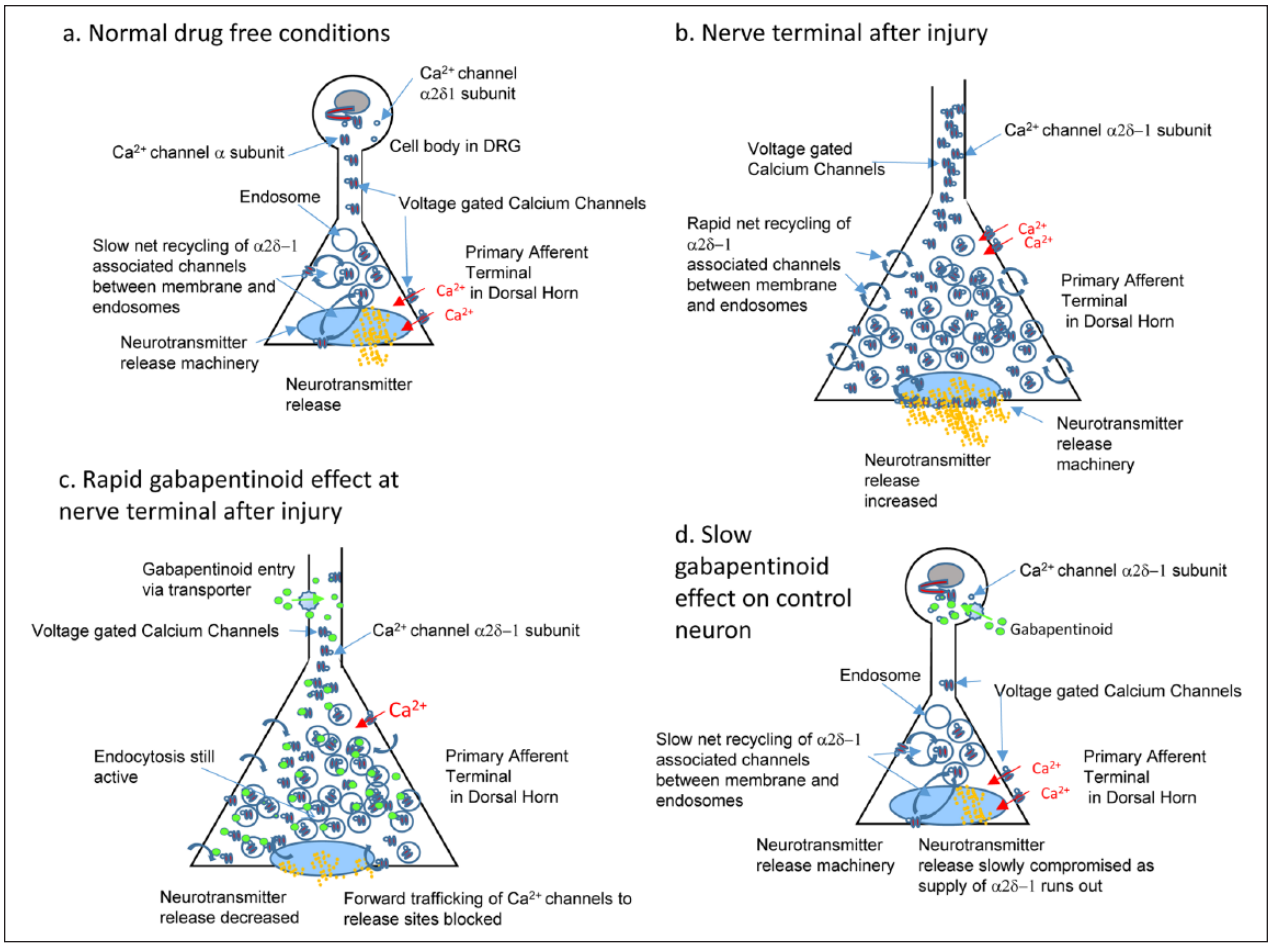

Figure 5. Schematic representation of a dorsal root ganglion neuron and its terminal in the spinal dorsal horn to explain rapid and slowly developing effects of gabapentinoids. (a) The α−subunits of high-voltage activated (HVA) Ca^{2+} channels associate with α2δ−1 subunits and both are trafficked to the nerve terminal. α2δ−1 is responsible for trafficking channels to the plasma membrane thereby setting the abundance of functional HVA-Ca^{2+} channels and enabling their interaction with the neurotransmitter release machinery. Expressed channels are removed from the membrane by endocytosis into endosomes where they are targeted for recycling or degradation. With low, physiological levels of α2δ−1, the recycling of Ca^{2+} channels proceeds relatively slowly. (b) Schematic representation of a primary afferent terminal when α2δ−1 levels are increased. The terminal becomes much more "busy," more Ca^{2+} channels may be targeted to the release machinery and neurotransmitter release is increased. The rate of channel turnover at the plasma membrane is assumed to be increased. (c) In the presence of gabapentinoids, the rapid forward trafficking of HVA Ca^{2+} to the plasma membrane and release sites is decreased. Interruption of this rapid process may account for rapid acute effects of gabapentinoids in situations where α2δ1 is up-regulated. (d) Diagram to illustrate the slowly developing actions of gabapentinoids seen in naïve animals. Impaired trafficking of α2δ−1 subunits and α−subunits gradually depletes them at release sites and neurotransmitter release declines over a period of many hours.

From the available literature, we suggest the following explanation. α2δ-1 subunits are complexed with pore forming α-subunits and accessory β-subunits in post-Golgi compartments of the endoplasmic reticulum and Golgi apparatus of neuronal cell bodies (Canti and others 2005; Tran-Van-Minh and Dolphin 2010). In primary afferent terminals, channel complexes comprising α2δ-1, α-subunits, and β-subunits are transported and inserted into the plasma membrane (Heblich and others 2008). This enables plasma membrane expression of HVA-Ca^{2+} channels but more importantly, enables their coupling to the neurotransmitter release machinery (Hoppa and others 2012). The channel complexes are then removed from the plasma membrane by endocytosis (Bauer and others 2009; Dolphin 2012; Tran-Van-Minh and Dolphin 2010) into early endosomes where they are targeted for recycling or degradation. In the control or uninjured situation, where levels of α2δ-1 are low, cycling of protein to and from the plasma membrane in nerve terminals may be a relatively slow overall process (Fig. 5a). However, this process may become much more rapid when α2δ-1 is up-regulated either experimentally or as result of nerve

injury as new protein is inserted into the plasma membrane (Tran-Van-Minh and Dolphin 2010) (Fig. 5b). Since gabapentinoids do not appear to affect the rate of endocytosis (Tran-Van-Minh and Dolphin, 2010), this renders surface expression of α2δ-1 more labile and susceptible to inhibition by gabapentinoids, which may be capable of exerting their effects within minutes (Tran-Van-Minh and Dolphin 2010) rather than hours (Biggs and others 2014; Biggs and others 2015; Heblich and others 2008; Hendrich and others 2008) (Fig. 5c).

In uninjured nerves, where α2δ-1 is not up-regulated, gabapentinoid impediment of trafficking of α2δ-1–HVA-I_{Ca} complexes from cell bodies to nerve terminals (Bauer and others 2009) will cause gradual depletion of their surface expression as the rate of endocytosis exceeds the rate of replenishment (Fig. 5d); a processes that appears to take 17 hours or more to occur (Heblich and others 2008).

Recent data from our laboratory are consistent with this mechanism. We find gabapentinoids have limited acute effects on dorsal horn neurons in spinal cord slices isolated from sham-operated animals whereas profound suppression of synaptic transmission and excitability can be observed in neurons in slices from nerve injured animals (Alles and others 2015). Similar rapid effects of gabapentin on excitatory synaptic transmission in both deep dorsal horn neurons (Zhou and Luo 2015) and in superficial laminae (Zhou and Luo 2014) have been seen in α2δ-1-overexpressing transgenic mice. The drugs thus act slowly in non-injured neurons and rapidly in injured neurons.

An Analogy to Explain Gabapentinoid Action

Let us assume the axon from the cell body is analogous to a highway into a city, the nerve terminal is the city, cars are α2δ-1 subunits and gabapentinoids represent roadworks. Under normal conditions, roadworks on the highway will eventually decrease the number of cars getting to the city so the supply of cars will very slowly run out (slowly developing effects of gabapentinoids on uninjured nerves in vitro; Fig. 5d). Similarly, if there are roadworks in the city, late at night or a on a Sunday, this will not have much effect on the movements of the few cars (α2δ-1 subunits) that are active (limited acute effect of gabapentinoids in uninjured nerves). By contrast, at rush hour (equivalent to the nerve injury situation; Fig. 5b) where there are many more active α2δ-1 subunits in the nerve terminals (cars in the city), gabapentinoids (roadworks) will have a much more rapid and profound action (Fig. 5c). "Gridlock" may account for their rapid action in terminals after nerve injury. This analogy may also explain the rapid reversibility of gabapentinoid action in vivo (Yang and others 2014) (see also Fig. 4a). Removal of drug would alleviate the "gridlock" in the active terminal and allow the resumption of rapid cycling of α2δ-1 into the plasma membrane. Thus reenabling interaction of HVA-Ca^{2+} channels with neurotransmitter release sites.

Additional Factors that Contribute to the Rapid Action of Gabapentinoids In Vivo

In the preceding sections we have considered the actions of gabapentinoids in the dorsal horn as this is a major site of nociceptive processing. Because gabapentinoids are effective as anticonvulsants (Dworkin & Kirkpatrick 2005; Kondo and others 1991; Sivenius and others 1991) and anxiolytics (Singh and others 1996) they obviously exert actions, at many, if not all levels of the central nervous system. Such effects likely involve interactions with various types of α2δ subunit (Dolphin 2012). That rapid central effects may contribute to the overall anti-allodynic actions of gabapentinoids in vivo is supported by a study using 8-F-fluorodeoxyglucose–positron emission tomography (Lin and others 2014). These authors showed that spared nerve injury-induced increases of glucose metabolism in thalamus, cerebellar vermis, and medial prefrontal cortex and that these changes were attenuated by acute gabapentin treatment.

Gabapentinoids may also exert acute in vivo effects as a result of inhibition of descending serotonergic facilitation of nociceptive processing (Rahman and others 2009; Suzuki and others 2005). Such acute effects would obviously be absent in ex vivo spinal cord slices or in organotypic cultures yet would be present in vivo. Interestingly, this effect does not appear to involve effects of gabapentin on serotonin release but may rather include acute interaction and inhibition of $5HT_3$ receptors (Suzuki and others 2005). The precise mechanism and the possible role of α2δ in this interaction remains to be elucidated.

An additional central mechanism that would be absent in acutely isolated spinal cord slices relates to the observation that gabapentin acutely (30-90 minutes) increases glutamate levels in the locus coeruleus (Suto and others 2014). This reflects acute inhibition of the astroglial glutamate transporter (GLT-1/EAAT2). Since the locus coeruleus provides a descending inhibitory noradrenergic input to the dorsal horn (Tanabe and others 2005), this effect would be expected to reduce excitability and to impede the transfer of nociceptive information.

Since ectopic activity in damaged peripheral nerves is required to drive central sensitization at the level of the dorsal horn (Pitcher and Henry 2008; Vaso and others 2014) and there are reports of acute gabapentioid actions on peripheral nerves (Pan and others 1999; Yang and others 2009) such actions may also contribute to the appearance of rapid drug effects in vivo.

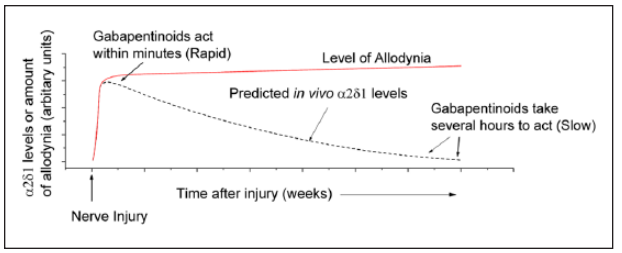

Figure 6. Scheme to illustrate predicted changes in α2δ-1 levels following nerve injury. In the days or weeks following injury, α2δ levels are increased and gabapentinoids act rapidly. Although allodynia may persist indefinitely after injury, α2δ-1 levels may return to control levels, this would predict slowly developing effects of gabapentinoids that may parallel the clinical situation.

The Role of α2δ as a Thrombospondin Receptor

Interestingly, α2δ-1, which is expressed extracellularly in mature Ca^{2+} channels (Fig. 2) (Dolphin 2012, 2013; Hendrich and others 2008), has been implicated as a receptor for a group of neurotrophins known as thrombospondins (Eroglu and others 2009; Risher and Eroglu 2012). All five members of this group (TSP 1-5) are secreted matrix proteins and all have been implicated in excitatory synaptogenesis (Christopherson and others 2005; Eroglu and others 2009). One member of this group, thrombospondin 4 (TSP4) has been implicated in the etiology of neuropathic pain (Kim and others 2012; Pan et al 2015). TSP4 is expressed in astrocytes and is up-regulated in the injury side of dorsal spinal cord and this correlates with the development of signs of neuropathic pain. TSP4 blockade by intrathecally delivered antibodies, antisense oligodeoxynucleotides, or inactivation of the TSP4 gene reverses or prevents behavioral hypersensitivity. Intrathecal injection of TSP4 protein into naive rats increases the frequency of mEPSCs in dorsal horn neurons (Kim and others 2012), suggesting an increased excitatory presynaptic input that would be consistent with behavioral hypersensitivity.

Seven days of gabapentinoid treatment has been shown to decrease synapse formation in cortical structures (Eroglu and others 2009). Since both neuropathic pain and α2δ subunits have been associated with excitatory synaptogenesis (Bauer and others 2010b; Crosby and others 2015; Li and others 2014), gabapentinoid interaction with α2δ-1 to antagonize the actions of thrombospondins may contribute to some of its more slowly developing effects. It is not, however, an exclusive mechanism for three reasons.

1. Astrocytes secrete TSPs to increase synapse number (Christopherson and others 2005) but we have seen slowly developing, neuron-subtype specific effects from in vitro experiments in neuron-enriched cultures of DRG neurons, which do not contain astrocytes (Biggs and others 2014). Thus, the presence of thrombospondin is not needed for gabapentinoid action. Data shown in Figure 3b were obtained from such cultures.
2. Since the effects of gabapentinoids are prevented following blockade of uptake into the neuronal cytoplasm by of 2-aminobicyclo-(2,2,1)-heptane-2-carboxylic acid (BCH) (Biggs and others 2014, 2015; Hendrich and others 2008), it would seem unlikely that they act exclusively at an extracellular binding site on mature HVA-Ca^{2+} channels.
3. Because actions of gabapentinoids can be observed within minutes of application under appropriate experimental conditions both in vivo (Coderre and others 2005; Kumar and others 2013; Narita and others 2012) and in vitro (Alles and others 2015; Zhou and Luo 2014, 2015) these effects are unlikely to reflect impairment of the slow process of synaptogenesis. This idea is supported by the observation of Kim and others (2012) that TSP 4 takes at least 4 days to increase synaptic transmission within the dorsal horn of the spinal cord.

Paradox 2: Rapid Effects in Animals but Slow Effects in People?

If it is accepted that the rapidity of onset of gabapentinoid action in vitro is directly related to the level of α2δ-1 expression and drug effects emerge within 30 minutes in animal models, why is it commonly reported that the drug effects take many days to appear in the clinic (Cheshire 2002; Gottrup and others 2004; Parsons and others 2015; Sharma and others 2010)? One possibility is that in patients presenting with chronic neuropathic pain, α2δ is no longer up-regulated and other maladaptive process have taken over the maintenance of central sensitization (Fig. 6). This idea is congruent with the likelihood that the processes that maintain neuropathic pain differ from those that initiate it. Thus, gabapentinoids may only act to slowly shut off the supply of α2δ subunits to nerve terminals in neuropathic pain patients. For example, when patients receive gabapentinoids to alleviate pain associated with diabetic or other neuropathies, there is no way of knowing when the initial precipitating nerve injury events occurred; they are in the "maintenance phase" of neuropathic pain. Our search of the literature revealed no information about the persistence of injury-induced α2δ up-regulation in either animal models or in patients. Such studies are urgently required to understand the protracted action of gabapentinoids in the clinic. Since gabapentinoids are not universally effective,

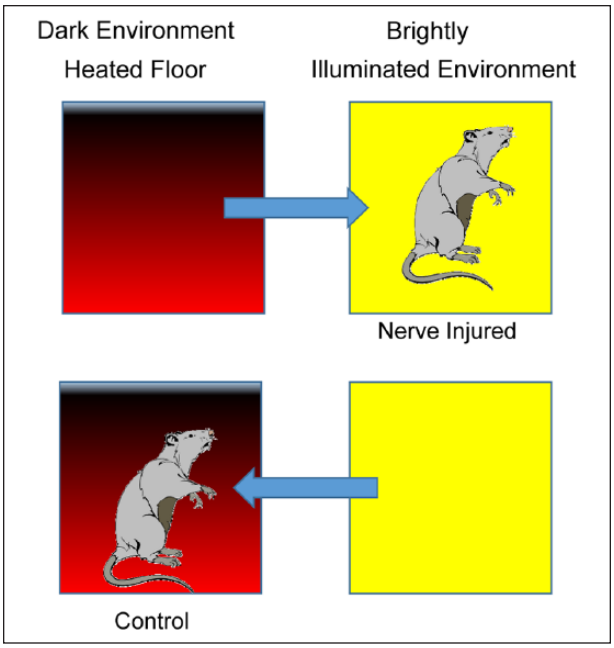

Figure 7. Diagram to illustrate an operant model for pain assessment in rodents. Normal rats will avoid an open, well-lit environment to avoid exposure to potential predators. If a darkened environment with a warm floor is provided, nerve injured rats will risk venturing into the open, well-lit environment to avoid thermal hyperalgesia they would experience in the dark. In other words, the animals have to decide whether they would prefer exposure to potential predators to avoid thermal hyperalgesia.

and as many as 50% of treated patients do not experience pain relief with gabapentin (Moore and others 2014), it would be interesting to know whether α2δ-1 levels in individual patients would predict drug efficacy.

Another likely factor relates to differences in measuring "pain" in animals versus people (Mogil 2009; Mogil and Crager 2004). Drug-induced increases in withdrawal threshold to tactile stimuli in neuropathic animals may simply reflect attenuation of spinal reflexes. For this reason, preclinical evaluations of anti-allodynic effectiveness are moving toward "operant" models (Xie and others 2014; Yezierski and others 2013). In this situation, the animal needs to make a decision based on the cortical processing of a noxious stimulus. For example, rats will naturally select for a covered, darkened environment to avoid predators. If a rat is nerve injured, a mildly warm stimulus will produce thermal hyperalgesia. In an operant test, the animal is given the choice of being on a warm surface in the dark or a cool surface in the light. If the animal is experiencing thermal hyperalgesia, it would be expected to spend more time in the light than in dark; a very different response from a naïve animal (see Fig. 7). To the best of our knowledge, relatively few studies of gabapentinoid action in operant pain models have been done (Munro and others 2007; Park and others 2015; Yezierski and others 2013). Further studies of this type may better align findings in animals with experience in the clinic.

There is also a good deal of interest in the use of gabapentinoids in post-surgical pain (Eipe and others 2015). In general, the effectiveness is rather variable but rapidly developing effects have been reported (Schmidt and others 2013). Our model posits that rapid effects of gabapentinoids will only occur under conditions where α2δ-1 is up-regulated. Interestingly, surgery itself (in the absence of deliberate nerve injury) has been reported to increase α2δ-1 (Bauer and others 2009) and it has also been suggested that injury-induced discharge in primary afferent fibers, as may occur during surgical manipulation, can up-regulate α2δ-1 (Boroujerdi and others 2008).

Detailed Actions of Gabapentinoids in the Dorsal Horn

Acute and slowly developing anti-allodynic effects of gabapentinoids involve attenuation of neurotransmitter release from primary afferent terminals and perhaps from other central and spinal sites (Biggs and others 2014; Coderre and others 2005; Moore and others 2002; Zhou and Luo 2015). Early studies on the effects of gabapentinoids did not address the effect on specific cell types within the spinal dorsal horn (Moore and others 2002). If gabapentinoids were to inhibit inhibitory neurons the resultant disinhibition would tend to increase overall excitability. This would be inconsistent with both their anti-allodynic action and their overall depressant effect on spinal cord excitability (Fig. 4c-e). Most tonic firing, low threshold, neurons in substantia gelatinosa exhibit a GABAergic phenotype and most high threshold, delay firing neurons are glutamatergic (Punnakkal and others 2014; Schoffnegger and others 2006; Yasaka and others 2010). In our study of long-term actions of pregabalin and gabapentin on substantia gelatinosa neurons in organotypic culture, we found that synaptic input to putative excitatory neurons was reduced preferentially (Biggs and others 2014) and this effect was only seen when drugs were present for 5 to 6 days. The basis of this nerve terminal selectivity of gabapentinoid action within the dorsal horn needs to be elucidated. One possibility may be that the excitatory terminals onto inhibitory substantia gelatinosa have reduced expression of the neutral amino acid transporter.

Gabapentinoids select for excitatory transmission in another way (Zhou and Luo 2015) as unlike mEPSCs, miniature IPSCs (inhibitory postsynaptic currents) in spinal neurons were unaffected by the drug. This finding is

congruent with the observation that α2δ-1 subunits preferentially localize with excitatory rather than inhibitory terminals in the spinal cord (Bauer and others 2009).

Conclusions

1. Although gabapentinoids are sometimes classified as "calcium channel blocking agents"; this does not really reflect their mechanism of action. They are more accurately described as drugs that depress neuronal excitability by a variety of mechanisms following their interaction with multifunctional α2δ proteins.
2. Both in the laboratory and in the clinic, some actions of gabapentinoids develop within less than 30 minutes whereas others take days or weeks to appear. It is suggested that the level of the gabapentinoid binding protein, α2δ-1 in nerve terminals directly dictates the rate of onset of gabapentinoid action in the laboratory and possibly within the clinic. This opens up the possibility for a personalized method of prescription of the gabapentinoids depending on α2δ-1 expression profile. It is possible that α2δ is no longer up-regulated in many patients who have endured neuropathic pain for periods of months or years. This may account for the 35% success rate observed with gabapentin in the clinical setting.

Acknowledgments

We thank Dr. Nataliya Bukhanova for carrying out the behavioral experiments illustrated in Figure 4a.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This study was supported by Pfizer Canada "Neuropathic Pain Research Awards" (Montreal, Quebec, Canada), Paralyzed Veterans of America (Washington, DC, USA) and Natural Sciences and Engineering Research Council (NSERC; Ottawa, Ontario, Canada).

References

Alles SRA, Bukhanova N, Bandet M, Winship IR, Smith PA. 2015. Peripheral nerve injury promotes emergence of acute, neuron-type-specific depressant actions of gabapentin in substantia gelatinosa and primary somatosensory cortex. Paper presented at: Neuroscience 2015; Society for Neuroscience Conference; Chicago, IL. Online Program No. 210.20.

Balasubramanyan S, Stemkowski PL, Stebbing MJ, Smith PA. 2006. Sciatic chronic constriction injury produces cell-type specific changes in the electrophysiological properties of rat substantia gelatinosa neurons. J Neurophysiol 96:579–90.

Baranauskas G, Nistri A. 1998. Sensitization of pain pathways in the spinal cord: cellular mechanisms. Prog Neurobiol 54:349–65.

Bauer CS, Nieto-Rostro M, Rahman W, Tran-Van-Minh A, Ferron L, Douglas L, and others. 2009. The increased trafficking of the calcium channel subunit $\alpha_2\delta$-1 to presynaptic terminals in neuropathic pain is inhibited by the $\alpha_2\delta$ ligand pregabalin. J Neurosci 29:4076–88.

Bauer CS, Rahman W, Tran-Van-Minh A, Lujan R, Dickenson AH, Dolphin AC. 2010a. The anti-allodynic $\alpha_2\delta$ ligand pregabalin inhibits the trafficking of the calcium channel $\alpha_2\delta$-1 subunit to presynaptic terminals in vivo. Biochem Soc Trans 38:525–8.

Bauer CS, Tran-Van-Minh A, Kadurin I, Dolphin AC. 2010b. A new look at calcium channel alpha2delta subunits. Curr Opin Neurobiol 20:563–71.

Berger JV, Knaepen L, Janssen SPM, Jaken RJP, Marcus MAE, Joosten EAJ, and others. 2011. Cellular and molecular insights into neuropathy-induced pain hypersensitivity for mechanism-based treatment approaches. Brain Res Rev 67:282–310.

Biggs JE, Boakye PA, Ganesan N, Stemkowski PL, Lantero A, Ballanyi K, and others. 2014. Analysis of the long-term actions of gabapentin and pregabalin in dorsal root ganglia and substantia gelatinosa. J Neurophysiol 112:2398–412.

Biggs JE, Lu VB, Kim H, Lai A, Todd KG, Ballanyi K, and others. 2012. Defined medium organotypic cultures of spinal cord put 'pain in a dish'. In: Ballanyi K, editor. Isolated brain circuits. New York, NY: Humana Press. pp. 405–35.

Biggs JE, Lu VB, Stebbing MJ, Balasubramanyan S, Smith PA. 2010. Is BDNF sufficient for information transfer between microglia and dorsal horn neurons during the onset of central sensitization? Mol Pain 6:44.

Biggs JE, Stemkowski PL, Knaus EE, Chowdhury MA, Ballanyi K, Smith PA. 2015. Suppression of network activity in dorsal horn by gabapentin permeation of TRPV1 channels; implications for drug access to cytoplasmic targets. Neurosci Lett 584:397-402.

Boroujerdi A, Kim HK, Lyu YS, Kim DS, Figueroa KW, Chung JM, and others. 2008. Injury discharges regulate calcium channel alpha-2-delta-1 subunit upregulation in the dorsal horn that contributes to initiation of neuropathic pain. Pain 139:358–66.

Boroujerdi A, Zeng J, Sharp K, Kim D, Steward O, Luo ZD. 2011. Calcium channel alpha-2-delta-1 protein upregulation in dorsal spinal cord mediates spinal cord injury-induced neuropathic pain states. Pain 152:649–55.

Canti C, Nieto-Rostro M, Foucault I, Heblich F, Wratten J, Richards MW, and others. 2005. The metal-ion-dependent adhesion site in the von Willebrand factor–A domain of

α2δ subunits is key to trafficking voltage-gated Ca^{2+} channels. Proc Natl Acad Sci U S A 102:11230–5.

Cao YQ, Tsien RW. 2010. Different relationship of N- and P/Q-type Ca^{2+} channels to channel-interacting slots in controlling neurotransmission at cultured hippocampal synapses. J Neurosci 30:4536–46.

Cassidy JS, Ferron L, Kadurin I, Pratt WS, Dolphin AC. 2014. Functional exofacially tagged N-type calcium channels elucidate the interaction with auxiliary α2δ-1 subunits. Proc Natl Acad Sci U S A 111:8979–84.

Chen Y, Balasubramanyan S, Lai AY, Todd KG, Smith PA. 2009. Effects of sciatic nerve axotomy on excitatory synaptic transmission in rat substantia gelatinosa. J Neurophysiol 102:3203–15.

Cheng JK, Chiou LC. 2006. Mechanisms of the antinociceptive action of gabapentin. J Pharmacol Sci 100:471–86.

Cheshire WP. 2002. Defining the role for gabapentin in the treatment of trigeminal neuralgia: a retrospective study. J Pain 3:137–42.

Christopherson KS, Ullian EM, Stokes CC, Mullowney CE, Hell JW, Agah A, and others. 2005. Thrombospondins are astrocyte-secreted proteins that promote CNS synaptogenesis. Cell 120:421–33.

Coderre TJ, Kumar N, Lefebvre CD, Yu JS. 2005. Evidence that gabapentin reduces neuropathic pain by inhibiting the spinal release of glutamate. J Neurochem 94:1131–9.

Costigan M, Scholz J, Woolf CJ. 2009. Neuropathic pain: a maladaptive response of the nervous system to damage. Annu Rev Neurosci 32:1-32.

Coull JA, Beggs S, Boudreau D, Boivin D, Tsuda M, Inoue K, and others. 2005. BDNF from microglia causes the shift in neuronal anion gradient underlying neuropathic pain. Nature 438:1017–21.

Coull JA, Boudreau D, Bachand K, Prescott SA, Nault F, Sik A, and others. 2003. Trans-synaptic shift in anion gradient in spinal lamina I neurons as a mechanism of neuropathic pain. Nature 424:938–42.

Crosby ND, Zaucke F, Kras JV, Dong L, Luo ZD, Winkelstein BA. 2015. Thrombospondin-4 and excitatory synaptogenesis promote spinal sensitization after painful mechanical joint injury. Exp Neurol 264:111–20.

Dalal A, Tata M, Allègre G, Gekiere F, Bons N, Albe-Fessard D. 1999. Spontaneous activity of rat dorsal horn cells in spinal segments of sciatic projection following transection of sciatic nerve or of corresponding dorsal roots. Neuroscience 94:217–28.

Davies A, Kadurin I, Alvarez-Laviada A, Douglas L, Nieto-Rostro M, Bauer CS, and others. 2010. The α2δ subunits of voltage-gated calcium channels form GPI-anchored proteins, a posttranslational modification essential for function. Proc Natl Acad Sci U S A 107:1654–9.

Decosterd I, Woolf CJ. 2000. Spared nerve injury: an animal model of persistent peripheral neuropathic pain. Pain 87:149–58.

Dib-Hajj SD, Cummins TR, Black JA, Waxman SG. 2010. Sodium channels in normal and pathological pain. Annu Rev Neurosci 33:325–47.

Dodge FA, Rahamimoff R. 1967. Co-operative action of calcium ions in transmitter release at the neuromuscular junction. J Physiol (Lond) 193:419–432.

Dolphin AC. 2012. Calcium channel auxiliary α2δ and β subunits: trafficking and one step beyond. Nat Rev Neurosci 13:542–55.

Dolphin AC. 2013. The α2δ subunits of voltage-gated calcium channels. Biochim Biophys Acta 1828:1541–9.

Dooley DJ, Taylor CP, Donevan S, Feltner D. 2007. Ca^{2+} channel $\alpha_2\delta$ ligands: novel modulators of neurotransmission. Trends Pharmacol Sci 28:75–82.

Dunlap K, Fischbach GD. 1981. Neurotransmitters decrease the calcium conductance activated by depolarization of embryonic chick sensory neurones. J Physiol (Lond) 317:519–35.

Dworkin RH, Kirkpatrick P. 2005. Pregabalin. Nat Rev Drug Discov 4:455–6.

Eipe N, Penning J, Yazdi F, Mallick R, Turner L, Ahmadzai N, and others. 2015. Perioperative use of pregabalin for acute pain—a systematic review and meta-analysis. Pain 156:1284–300.

Eroglu C, Allen NJ, Susman MW, O'Rourke NA, Park CY, Ozkan E, and others. 2009. Gabapentin receptor α2δ-1 is a neuronal thrombospondin receptor responsible for excitatory CNS synaptogenesis. Cell 139:380–92.

Fenselau H, Heinke B, Sandkuhler J. 2011. Heterosynaptic long-term potentiation at GABAergic synapses of spinal lamina I neurons. J Neurosci 31:17383–91.

Field MJ, Cox PJ, Stott E, Melrose H, Offord J, Su TZ, and others. 2006. Identification of the α2δ-1 subunit of voltage-dependent calcium channels as a molecular target for pain mediating the analgesic actions of pregabalin. Proc Natl Acad Sci U S A 103:17537–42.

Fink K, Meder W, Dooley DJ, Gothert M. 2000. Inhibition of neuronal Ca^{2+} influx by gabapentin and subsequent reduction of neurotransmitter release from rat neocortical slices. Br J Pharmacol 130:900–6.

Finnerup NB, Attal N, Haroutounian S, McNicol E, Baron R, Dworkin RH, and others. 2015. Pharmacotherapy for neuropathic pain in adults: a systematic review and meta-analysis. Lancet Neurol 14:162–73.

Fox A, Gentry C, Patel S, Kesingland A, Bevan S. 2003. Comparative activity of the anti-convulsants oxcarbazepine, carbamazepine, lamotrigine and gabapentin in a model of neuropathic pain in the rat and guinea-pig. Pain 105:355–62.

Gee NS, Brown JP, Dissanayake VU, Offord J, Thurlow R, Woodruff GN. 1996. The novel anticonvulsant drug, gabapentin (Neurontin), binds to the α2δ subunit of a calcium channel. J Biol Chem 271:5768–76.

Gilron I, Watson CP, Cahill CM, Moulin DE. 2006. Neuropathic pain: a practical guide for the clinician. CMAJ 175:265–75.

Gottrup H, Juhl G, Kristensen AD, Lai R, Chizh BA, Brown J, and others. 2004. Chronic oral gabapentin reduces elements of central sensitization in human experimental hyperalgesia. Anesthesiology 101:1400–8.

Grace PM, Hutchinson MR, Maier SF, Watkins LR. 2014. Pathological pain and the neuroimmune interface. Nat Rev Immunol 14:217–31.

Gruber-Schoffnegger D, Drdla-Schutting R, Honigsperger C, Wunderbaldinger G, Gassner M, Sandkuhler J. 2013. Induction of thermal hyperalgesia and synaptic long-term potentiation in the spinal cord lamina I by TNF-α and IL-1β is mediated by glial cells. J Neurosci 33:6540–51.

Gustin SM, Peck CC, Cheney LB, Macey PM, Murray GM, Henderson LA. 2012. Pain and plasticity: is chronic pain always associated with somatosensory cortex activity and reorganization? J Neurosci 32:14874–84.

Heblich F, Tran Van Minh A, Hendrich J, Watschinger K, Dolphin AC. 2008. Time course and specificity of the pharmacological disruption of the trafficking of voltage-gated calcium channels by gabapentin. Channels (Austin) 2:4-9.

Hendrich J, Bauer CS, Dolphin AC. 2012. Chronic pregabalin inhibits synaptic transmission between rat dorsal root ganglion and dorsal horn neurons in culture. Channels (Austin) 6:124–32.

Hendrich J, Van Minh AT, Heblich F, Nieto-Rostro M, Watschinger K, Striessnig J, and others. 2008. Pharmacological disruption of calcium channel trafficking by the α2δ ligand gabapentin. Proc Natl Acad Sci U S A 105:3628–33.

Hoppa MB, Lana B, Margas W, Dolphin AC, Ryan TA. 2012. α2δ expression sets presynaptic calcium channel abundance and release probability. Nature 486:122–5.

Kim DS, Li KW, Boroujerdi A, Peter YY, Zhou CY, Deng P, and others. 2012. Thrombospondin-4 contributes to spinal sensitization and neuropathic pain states. J Neurosci 32:8977–87.

Kim KJ, Yoon YW, Chung JM. 1997. Comparison of three rodent models of neuropathic pain. Exp Brain Res 113:200–6.

Kondo T, Fromm GH, Schmidt B. 1991. Comparison of gabapentin with other antiepileptic and GABAergic drugs. Epilepsy Res 8:226–31.

Kumar N, Cherkas PS, Varathan V, Miyamoto M, Chiang CY, Dostrovsky JO, and others. 2013. Systemic pregabalin attenuates facial hypersensitivity and noxious stimulus-evoked release of glutamate in medullary dorsal horn in a rodent model of trigeminal neuropathic pain. Neurochem Int 62:831–5.

Kushnir MM, Crossett J, Brown PI, Urry FM. 1999. Analysis of gabapentin in serum and plasma by solid-phase extraction and gas chromatography-mass spectrometry for therapeutic drug monitoring. J Anal Toxicol 23:1-6.

Lacinova L, Klugbauer N, Hofmann F. 2000. Low voltage activated calcium channels: from genes to function. Gen Physiol Biophys 19:121–36.

Lanneau C, Green A, Hirst WD, Wise A, Brown JT, Donnier E, and others. 2001. Gabapentin is not a GABAB receptor agonist. Neuropharmacology 41:965–75.

Lee KY, Prescott SA. 2015. Chloride dysregulation and inhibitory receptor blockade yield equivalent disinhibition of spinal neurons yet are differentially reversed by carbonic anhydrase blockade. Pain 156:2431–7.

Leitner J, Westerholz S, Heinke B, Forsthuber L, Wunderbaldinger G, Jager T, and others. 2013. Impaired excitatory drive to spinal GABAergic neurons of neuropathic mice. PLoS One 8:e73370.

Li CY, Zhang XL, Matthews EA, Li KW, Kurwa A, Boroujerdi A, and others. 2006. Calcium channel alpha2delta1 subunit mediates spinal hyperexcitability in pain modulation. Pain 125:20–34.

Li KW, Yu YP, Zhou C, Kim DS, Lin B, Sharp K, and others. 2014. Calcium channel α2δ-1 proteins mediate trigeminal neuropathic pain states associated with aberrant excitatory synaptogenesis. J Biol Chem 289:7025–37.

Li Z, Taylor CP, Weber M, Piechan J, Prior F, Bian F, and others. 2011. Pregabalin is a potent and selective ligand for $\alpha_2\delta$-1 and $\alpha_2\delta$-2 calcium channel subunits. Eur J Pharmacol 667:80-90.

Lin HC, Huang YH, Chao TH, Lin WY, Sun WZ, Yen CT. 2014. Gabapentin reverses central hypersensitivity and suppresses medial prefrontal cortical glucose metabolism in rats with neuropathic pain. Mol Pain 10:63.

Lu VB, Biggs JE, Stebbing MJ, Balasubramanyan S, Todd KG, Lai AY, and others. 2009. BDNF drives the changes in excitatory synaptic transmission in the rat superficial dorsal horn that follow sciatic nerve injury. J Physiol (Lond) 587:1013–32.

Lu VB, Moran TD, Balasubramanyan S, Alier KA, Dryden WF, Colmers WF, and others. 2006. Substantia gelatinosa neurons in defined-medium organotypic slice culture are similar to those in acute slices from young adult rats. Pain 121:261–75.

Luo ZD, Calcutt NA, Higuera ES, Valder CR, Song YH, Svensson CI, and others. 2002. Injury type-specific calcium channel α2δ-1 subunit up-regulation in rat neuropathic pain models correlates with antiallodynic effects of gabapentin. J Pharmacol Exp Ther 303:1199–205.

Mack A. 2003. Examination of the evidence for off-label use of gabapentin. J Manag Care Pharm 9:559–68.

Martinotti G, Lupi M, Sarchione F, Santacroce R, Salone A, De BD, and others. 2013. The potential of pregabalin in neurology, psychiatry and addiction: a qualitative overview. Curr Pharm Des 19:6367–74.

Masocha W. 2015. Astrocyte activation in the anterior cingulate cortex and altered glutamatergic gene expression during paclitaxel-induced neuropathic pain in mice. PeerJ 3:e1350.

McClelland D, Evans RM, Barkworth L, Martin DJ, Scott RH. 2004. A study comparing the actions of gabapentin and pregabalin on the electrophysiological properties of cultured DRG neurones from neonatal rats. BMC Pharmacol 4:14.

Mogil JS. 2009. Animal models of pain: progress and challenges. Nat Rev Neurosci 10:283–94.

Mogil JS, Crager SE. 2004. What should we be measuring in behavioral studies of chronic pain in animals? Pain 112:12–15.

Moore KA, Baba H, Woolf CJ. 2002. Gabapentin—actions on adult superficial dorsal horn neurons. Neuropharmacology 43:1077–81.

Moore RA, Wiffen PJ, Derry S, Toelle T, Rice AS. 2014. Gabapentin for chronic neuropathic pain and fibromyalgia in adults. Cochrane Database Syst Rev 4:CD007938.

Moulin D, Boulanger A, Clark AJ, Clarke H, Dao T, Finley GA, and others. 2014. Pharmacological management of chronic neuropathic pain: revised consensus statement from the Canadian Pain Society. Pain Res Manag 19:328–35.

Moulin DE, Clark AJ, Gilron I, Ware MA, Watson CP, Sessle BJ, and others. 2007. Pharmacological management of

chronic neuropathic pain - consensus statement and guidelines from the Canadian Pain Society. Pain Res Manag 12:13-21.

Munro G, Erichsen HK, Mirza NR. 2007. Pharmacological comparison of anticonvulsant drugs in animal models of persistent pain and anxiety. Neuropharmacology 53: 609–18.

Narita N, Kumar N, Cherkas PS, Chiang CY, Dostrovsky JO, Coderre TJ, and others. 2012. Systemic pregabalin attenuates sensorimotor responses and medullary glutamate release in inflammatory tooth pain model. Neuroscience 218:359–66.

Pan B, Yu H, Park J, Yu YP, Luo ZD, Hogan QH. 2015. Painful nerve injury upregulates thrombospondin-4 expression in dorsal root ganglia. J Neurosci Res 93:443–53.

Pan HL, Eisenach JC, Chen SR. 1999. Gabapentin suppresses ectopic nerve discharges and reverses allodynia in neuropathic rats. J Pharmacol Exp Ther 288:1026–30.

Park HJ, Sandor K, McQueen J, Woller SA, Svensson CI, Corr M, and others. 2015. The effect of gabapentin and ketorolac on allodynia and conditioned place preference in antibody-induced inflammation. Eur J Pain. Epub ahead of print. Oct 30. doi:10.1002/ejp.816.

Parsons B, Emir B, Clair A. 2015. Temporal analysis of pain responders and common adverse events: when do these first appear following treatment with pregabalin. J Pain Res 8:303–9.

Patel MK, Gonzalez MI, Bramwell S, Pinnock RD, Lee K. 2000. Gabapentin inhibits excitatory synaptic transmission in the hyperalgesic spinal cord. Br J Pharmacol 130:1731–4.

Patel R, Bauer CS, Nieto-Rostro M, Margas W, Ferron L, Chaggar K, and others. 2013. α2δ-1 gene deletion affects somatosensory neuron function and delays mechanical hypersensitivity in response to peripheral nerve damage. J Neurosci 33:16412–26.

Patel S, Naeem S, Kesingland A, Froestl W, Capogna M, Urban L, and others. 2001. The effects of GABA(B) agonists and gabapentin on mechanical hyperalgesia in models of neuropathic and inflammatory pain in the rat. Pain 90:217–26.

Pitcher GM, Henry JL. 2008. Governing role of primary afferent drive in increased excitation of spinal nociceptive neurons in a model of sciatic neuropathy. Exp Neurol 214:219–28.

Punnakkal P, von Schoultz C, Haenraets K, Wildner H, Zeilhofer HU. 2014. Morphological, biophysical and synaptic properties of glutamatergic neurons of the mouse spinal dorsal horn. J Physiol 592:759–76.

Rahman W, Bauer CS, Bannister K, Vonsy JL, Dolphin AC, Dickenson AH. 2009. Descending serotonergic facilitation and the antinociceptive effects of pregabalin in a rat model of osteoarthritic pain. Mol Pain 5:45.

Rettig J, Sheng ZH, Kim DK, Hodson CD, Snutch TP, Catterall WA. 1996. Isoform-specific interaction of the α1A subunits of brain Ca^{2+} channels with the presynaptic proteins syntaxin and SNAP-25. Proc Natl Acad Sci U S A 93:7363–8.

Risher WC, Eroglu C. 2012. Thrombospondins as key regulators of synaptogenesis in the central nervous system. Matrix Biol 31:170–7.

Ruscheweyh R, Wilder-Smith O, Drdla R, Liu XG, Sandkuhler J. 2011. Long-term potentiation in spinal nociceptive pathways as a novel target for pain therapy. Mol Pain 7:20.

Sandkuhler J. 2009. Models and mechanisms of hyperalgesia and allodynia. Physiol Rev 89:707–58.

Sandkuhler J, Gruber-Schoffnegger D. 2012. Hyperalgesia by synaptic long-term potentiation (LTP): an update. Curr Opin Pharmacol 12:18–27.

Schmidt PC, Ruchelli G, Mackey SC, Carroll IR. 2013. Perioperative gabapentinoids: choice of agent, dose, timing, and effects on chronic postsurgical pain. Anesthesiology 119:1215–21.

Schoffnegger D, Heinke B, Sommer C, Sandkuhler J. 2006. Physiological properties of spinal lamina II GABAergic neurons in mice following peripheral nerve injury. J Physiol 577:869–78.

Sharma U, Griesing T, Emir B, Young JP, Jr. 2010. Time to onset of neuropathic pain reduction: a retrospective analysis of data from nine controlled trials of pregabalin for painful diabetic peripheral neuropathy and postherpetic neuralgia. Am J Ther 17:577–85.

Sheng ZH, Rettig J, Takahashi M, Catterall WA. 1994. Identification of a syntaxin-binding site on N-type calcium channels. Neuron 13:1303–13.

Simms BA, Zamponi GW. 2014. Neuronal voltage-gated calcium channels: structure, function, and dysfunction. Neuron 82:24–45.

Sindrup SH, Jensen TS. 1999. Efficacy of pharmacological treatments of neuropathic pain: an update and effect related to mechanism of drug action. Pain 83:389–400.

Singh L, Field MJ, Ferris P, Hunter JC, Oles RJ, Williams RG, and others. 1996. The antiepileptic agent gabapentin (Neurontin) possesses anxiolytic-like and antinociceptive actions that are reversed by D-serine. Psychopharmacology (Berl) 127:1-9.

Sivenius J, Kalviainen R, Ylinen A, Riekkinen P. 1991. Double-blind study of gabapentin in the treatment of partial seizures. Epilepsia 32:539–42.

Stoicea N, Russell D, Weidner G, Durda M, Joseph NC, Yu J, and others. 2015. Opioid-induced hyperalgesia in chronic pain patients and the mitigating effects of gabapentin. Front Pharmacol 6:104.

Su TZ, Lunney E, Campbell G, Oxender DL. 1995. Transport of gabapentin, a gamma-amino acid drug, by system l α-amino acid transporters: a comparative study in astrocytes, synaptosomes, and CHO cells. J Neurochem 64:2125–31.

Suto T, Severino AL, Eisenach JC, Hayashida K. 2014. Gabapentin increases extracellular glutamatergic level in the locus coeruleus via astroglial glutamate transporter-dependent mechanisms. Neuropharmacology 81:95–100.

Sutton KG, Martin DJ, Pinnock RD, Lee K, Scott RH. 2002. Gabapentin inhibits high-threshold calcium channel currents in cultured rat dorsal root ganglion neurones. Br J Pharmacol 135:257–65.

Suzuki R, Rahman W, Rygh LJ, Webber M, Hunt SP, Dickenson AH. 2005. Spinal-supraspinal serotonergic circuits regulating neuropathic pain and its treatment with gabapentin. Pain 117:292–303.

Tanabe M, Takasu K, Kasuya N, Shimizu S, Honda M, Ono H. 2005. Role of descending noradrenergic system and spinal α2-adrenergic receptors in the effects of gabapentin on thermal and mechanical nociception after partial nerve injury in the mouse. Br J Pharmacol 144:703–14.

Taylor CP, Angelotti T, Fauman E. 2007. Pharmacology and mechanism of action of pregabalin: the calcium channel α_2-δ (alpha$_2$-delta) subunit as a target for antiepileptic drug discovery. Epilepsy Res 73:137–50.

Taylor RS. 2006. Epidemiology of refractory neuropathic pain. Pain Pract 6:22–6.

Torrance N, Ferguson JA, Afolabi E, Bennett MI, Serpell MG, Dunn KM, and others. 2013. Neuropathic pain in the community: more under-treated than refractory? Pain 154:690–9.

Torrance N, Smith BH, Bennett MI, Lee AJ. 2006. The epidemiology of chronic pain of predominantly neuropathic origin. Results from a general population survey. J Pain 7:281–9.

Tran-Van-Minh A, Dolphin AC. 2010. The α2δ ligand gabapentin inhibits the Rab11-dependent recycling of the calcium channel subunit α2δ-2. J Neurosci 30:12856–67.

Tzellos TG, Toulis KA, Goulis DG, Papazisis G, Zampeli VA, Vakfari A, and others. 2010. Gabapentin and pregabalin in the treatment of fibromyalgia: a systematic review and a meta-analysis. J Clin Pharm Ther 35:639–56.

Vaso A, Adahan HM, Gjika A, Zahaj S, Zhurda T, Vyshka G, and others. 2014. Peripheral nervous system origin of phantom limb pain. Pain 155:1384–91.

von Hehn CA, Baron R, Woolf CJ. 2012. Deconstructing the neuropathic pain phenotype to reveal neural mechanisms. Neuron 73:638–52.

Wall PD, Devor M. 1983. Sensory afferent impulses result from dorsal root ganglia as well as from the periphery in normal and nerve-injured rats. Pain 17:321–39.

Waxman SG, Zamponi GW. 2014. Regulating excitability of peripheral afferents: emerging ion channel targets. Nat Neurosci 17:153–63.

Westenbroek RE, Hell JW, Warner C, Dubel SJ, Snutch TP, Catterall WA. 1992. Biochemical properties and subcellular distribution of an N-type calcium channel α1 subunit. Neuron 9:1099–115.

Whittaker CA, Hynes RO. 2002. Distribution and evolution of von Willebrand/integrin A domains: widely dispersed domains with roles in cell adhesion and elsewhere. Mol Biol Cell 13:3369–87.

Woolf CJ. 1983. Evidence for a central component of post-injury pain hypersensitivity. Nature 306:686–8.

Xie JY, Qu C, Patwardhan A, Ossipov MH, Navratilova E, Becerra L, and others. 2014. Activation of mesocorticolimbic reward circuits for assessment of relief of ongoing pain: a potential biomarker of efficacy. Pain 155:1659–66.

Xu H, Wu LJ, Wang H, Zhang X, Vadakkan KI, Kim SS, and others. 2008. Presynaptic and postsynaptic amplifications of neuropathic pain in the anterior cingulate cortex. J Neurosci 28:7445–53.

Yang F, Whang J, Derry WT, Vardeh D, Scholz J. 2014. Analgesic treatment with pregabalin does not prevent persistent pain after peripheral nerve injury in the rat. Pain 155:356–66.

Yang RH, Wang WT, Chen JY, Xie RG, Hu SJ. 2009. Gabapentin selectively reduces persistent sodium current in injured type-A dorsal root ganglion neurons. Pain 143:48-55.

Yasaka T, Tiong SY, Hughes DI, Riddell JS, Todd AJ. 2010. Populations of inhibitory and excitatory interneurons in lamina II of the adult rat spinal dorsal horn revealed by a combined electrophysiological and anatomical approach. Pain 151:475–88.

Yezierski RP, Green M, Murphy K, Vierck CJ. 2013. Effects of gabapentin on thermal sensitivity following spinal nerve ligation or spinal cord compression. Behav Pharmacol 24:598–609.

Zamponi GW. 2015. Calcium channel signaling complexes with receptors and channels. Curr Mol Pharmacol 8:8–11.

Zamponi GW. 2016. Targeting voltage-gated calcium channels in neurological and psychiatric diseases. Nat Rev Drug Discov 15:19–34. doi:10.1038/nrd.2015.5.

Zamponi GW, Striessnig J, Koschak A, Dolphin AC. 2015. The physiology, pathology, and pharmacology of voltage-gated calcium channels and their future therapeutic potential. Pharmacol Rev 67:821–70.

Zhang J, de Koninck Y. 2006. Spatial and temporal relationship between monocyte chemoattractant protein-1 expression and spinal glial activation following peripheral nerve injury. J Neurochem 97:772–83.

Zhou C, Luo ZD. 2014. Electrophysiological characterization of spinal neuron sensitization by elevated calcium channel α-2-δ-1 subunit protein. Eur J Pain 18:649–58.

Zhou C, Luo ZD. 2015. Nerve injury-induced calcium channel α-2-δ-1 protein dysregulation leads to increased presynaptic excitatory input into deep dorsal horn neurons and neuropathic allodynia. Eur J Pain 19:1267–76. doi:10.1002/ejp.656.

Zhuo M. 2008. Cortical excitation and chronic pain. Trends Neurosci 31:199–207.