

Acute modulation of calcium currents and synaptic transmission by gabapentinoids

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Abbreviations: GBPs, gabapentinoids; MNTB, medial nucleus of the trapezoid body; mEPPs, miniature endplate potentials; EPPs, endplate potentials; EPSC, excitatory post synaptic currents; VGCC, voltage-gated calcium channels; I_{pCa} , presynaptic calcium currents; EPSCs, excitatory postsynaptic currents; DRG, dorsal root ganglion

Gabapentin and pregabalin are anticonvulsant drugs that are extensively used for the treatment of several neurological and psychiatric disorders. Gabapentinoids (GBPs) are known to have a high affinity binding to $\alpha_2\delta$ -1 and $\alpha_2\delta$ -2 auxiliary subunit of specific voltage-gated calcium channels. Despite the confusing effects reported on Ca^{2+} currents, most of the studies showed that GBPs reduced release of various neurotransmitters from synapses in several neuronal tissues. We showed that acute in vitro application of pregabalin could reduce in a dose dependent manner synaptic transmission in both neuromuscular junctions and calyx of Held-MNTB excitatory synapses. Furthermore presynaptic Ca^{2+} currents treated with pregabalin are reduced in amplitude, do not show inactivation at a clinically relevant low concentration of 100 μ M and activate and deactivate faster. These results suggest novel modulatory role of acute pregabalin that might contribute to better understanding its anticonvulsant/analgesic clinical effects.

Introduction

Gabapentin (brand name Neurontin, (1-aminomethyl-cyclohexyl)-acetic acid), a substituted γ -amino acid, and its more potent successor Pregabalin (brand name Lyrica, S, -3-(aminomethyl)-5-methylhexanoic acid) are anticonvulsant drugs used in several neurological and psychiatric disorders including fibromyalgia, generalized anxiety disorder and as an adjunctive therapy in adults with partial seizures.¹ Both compounds are part of a family of GABA analog drugs generically called Gabapentinoids (GBPs). They were synthesized to mimic the chemical structure of the inhibitory neurotransmitter γ -aminobutyric acid (GABA) with the goal of increasing its lipophilicity to improve its penetration into the central nervous system while retaining a similar pharmacology. However, it is

accepted that they do not act on the GABA receptors. They are not converted to GABA or a GABA agonist and they are not an inhibitors of GABA uptake or degradation.^{2,3} Both compounds also differ from GABA because they readily cross membrane barriers via L-amino acid transporters.⁴ Additional substances, identified by their inhibitory potencies in [³H]-GBP-binding assays, have also been described in the literature.⁵

Multiple mechanisms of action have been proposed to account for the effects of these drugs.^{2,6} However, the mechanisms of action of the antiepileptic and antinociceptive drugs of the gabapentinoid family have remained poorly understood. In this review, we summarize and discuss the recent findings that help to better understand the mechanisms of action of the acute application of GBPs on synaptic transmission in peripheral and central nervous system.

Ligand Binding Site

Research conducted over the last decades has shown that GBPs exert their pharmacological effects through their high-affinity binding to the $\alpha_2\delta$ auxiliary subunit of specific voltage-gated calcium channels (VGCC).^{5,7-9}

The pharmacological target of gabapentin was initially found by studying the binding of [³H]-gabapentin in rat brain membranes¹⁰ (K_d ~40 nM) and the autoradiographic labeling of brain tissue.¹¹ In addition to the brain, [³H]-gabapentin binds to skeletal muscle tissue although no skeletal or cardiac muscle function is altered by GBPs.^{7,12} Fractionation of solubilized brain membranes identified the [³H]-gabapentin receptor as the $\alpha_2\delta$ subunit of calcium channels.⁷

VGCC are transmembrane proteins that play key roles throughout the body in controlling muscle contraction, neurotransmitter and hormone release, gene expression and cellular differentiation. Calcium channels comprise a series of polypeptides including the principal α_1 subunit, as well as β and $\alpha_2\delta$ auxiliary subunits in a 1:1:1 stoichiometry.¹³ Mammalian genes encoding ten α_1 subunits, four $\alpha_2\delta$ subunits and four β subunits have been identified (reviewed in ref. 14). Calcium channel heterogeneity is generated by distinct assembly profiles between these subunits and/or by alternatively spliced variants.¹⁵ The

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skeletal muscle calcium channel complex identified from initial purification studies is also composed by the γ subunit but this subunit does not appear to be part of the brain calcium channel complex.^{16,17}

The α_1 subunit contains 24 transmembrane domains, where the voltage sensor and ion channel are located. This subunit determines the main biophysical and pharmacological properties of the channel. The β subunit is a peripheral membrane protein that interacts with a cytoplasmic loop from the α_1 subunit (reviewed in ref. 18). The $\alpha_2\delta$ polypeptide is post-translationally cleaved to form α_2 and δ subunits which are both heavily glycosylated and are linked together by a disulfide bond. It was considered that the δ subunit spans the membrane once and binds to the extracellular α_2 subunit.¹⁹ However there is new evidence that the δ subunit may in fact be a glycosylphosphatidylinositol (GPI)-anchored extracellular protein.²⁰

The auxiliary $\alpha_2\delta$ and β subunits dramatically enhance trafficking of the principal α subunit to the cell surface.²¹ Also, these auxiliary subunits modify calcium channel gating.²³ The $\alpha_2\delta$ subunit accelerates channel activation and inactivation and exerts a hyperpolarizing shift in the current-voltage relationship.^{21,24}

Of the four known subtypes of $\alpha_2\delta$, only $\alpha_2\delta$ -1 and $\alpha_2\delta$ -2, but not $\alpha_2\delta$ -3 or $\alpha_2\delta$ -4, bind pregabalin and gabapentin.^{7,25,26} A structural domain in the α_2 subunits was identified by sequence homology as a von Willebrand factor type A domain, a site known to mediate a divalent cation dependent interaction with extracellular matrix proteins.²⁷ The $\alpha_2\delta$ -3 or $\alpha_2\delta$ -4 subunits do not contain the motif that is the key to gabapentin binding.^{7,25,26} The binding affinity of [³H]-gabapentin for $\alpha_2\delta$ is enhanced several folds in lipid rafts²⁸ and after removing endogenous small molecules ligands such as L-leucine or L-isoleucine which might normally occupy the site to which gabapentin binds.^{29,30}

Further proof that $\alpha_2\delta$ mediates the effects of gabapentin was derived from targeted point mutations in the $\alpha_2\delta$ subunit. A single amino acid substitution of the arginine residue at position 217 with alanine (R217A) on the $\alpha_2\delta$ -1 protein reduced [³H]-gabapentin binding in-vitro²⁵ and in a strain of genetically modified mice homozygous for the mutation.³¹ Saturation binding and autoradiography studies in R217A mice demonstrated a pronounced decrease in [³H]-gabapentin binding to areas where $\alpha_2\delta$ -1 is preferentially expressed (neocortex, hippocampus, basolateral amygdala and spinal cord), but little change occurs in binding to cerebellum and brainstem, which are dominated by $\alpha_2\delta$ -2.³² Additionally, analgesia from pregabalin, but not from opiates or tricyclic antidepressants, was abolished in the R217A knock in mice indicating that binding to the $\alpha_2\delta$ -1 subtype is required to achieve pain relief.³¹

Competition with the Gabapentinoid Binding Site: L-Isoleucine

Given the close structural resemblance between some endogenous amino acids (i.e., L-leucine, L-isoleucine) and $\alpha_2\delta$ drugs (i.e., GBPs), these amino acids might be considered as key tools to analyze the specificity of action of GBPs. L-Isoleucine and pregabalin have approximately equal affinity to the same binding

site (the $\alpha_2\delta$ subunit of VGCCs)³⁰ and both are substrates for the L-amino acid transporter system. Several in-vitro studies have used this amino acid to revert the effect of GBPs and when L-isoleucine is applied together with gabapentin or pregabalin, it reduces the activity of both compounds.³³ Some examples of these studies are the following: (1) L-isoleucine blocks gabapentin-mediated decrease of evoked and miniature excitatory postsynaptic potentials in rat entorhinal cortex slices;³⁴ (2) L-isoleucine blocks the action of pregabalin on vesicle release³⁵ at cultured hippocampal neurons; (3) L-Isoleucine but not D-Isoleucine, blocked the inhibitions of K(+)-evoked [³H]-noradrenalin on the release of neurotransmitters in human neocortical slices;³⁶ (4) L-isoleucine antagonized K(+)-evoked release of [³H]-GABA and [³H]-glutamate from superfused human neocortical synaptosomes;³⁷ (5) the presynaptic calcium current inhibited by PGB at the calyx of Held was partially recovered by L-Isoleucine.³⁸ Furthermore, these amino acids are present in human cerebrospinal fluid and could consequently modulate presynaptic function or alter activities that would otherwise occur with $\alpha_2\delta$ drugs, including direct competition with GBPs and a reduction of their potency.³⁰

GBP Interaction with Other Sites

Although compelling evidence indicates that $\alpha_2\delta$ subunits are major binding proteins for pregabalin in spinal cord and other brain areas it remains to be determined whether an interaction with high-voltage-activated calcium channels is sufficient to account for the broad-spectrum of activities of gabapentin and pregabalin.

L-amino acid transporter. An intracellular action of pregabalin mediated by the L-amino acid transporter has been suggested, which would be responsible for the drug absorption through the gastrointestinal tract and for its distribution after crossing the brain blood barrier.³⁹ However L-Leucine did not affect the antinociceptive effects of intrathecal administration of gabapentin in a pain model.⁴⁰ The fact that some gabapentin analogues that did not bind to the L-amino acid transporter had an anticonvulsant effect in-vivo via intraventricular application (but not orally) indicates that the L-amino acid transporter may be important to activate the transport of GBPs through the blood brain barrier.⁴¹

Neurotransmitter receptors. The effects of GBPs on inhibitory (GABA and glycine) and excitatory (N-methyl-D-aspartate (NMDA) and non-NMDA) amino acid neurotransmitter receptors were studied in cultured rodent neurons using intracellular, whole cell or single channel recording techniques. Gabapentin did not have a significant effect in any condition when tested at or above therapeutic concentrations for humans.⁴² However, more recent patch-clamp studies revealed that gabapentin significantly inhibited the NMDA receptor-activated ion current in dissociated hippocampal CA1 neurons. These results show that gabapentin may exert protective effects against glutamate-induced neuronal injury at least in part by inhibiting the NMDA receptor-activated ion current.⁴³

It has also been proposed that gabapentin acts as an agonist on a subtype of GABA (B) receptors.⁴⁴⁻⁴⁶ However this was not

confirmed using binding assays and searching for agonist effects on hippocampal slices.⁴⁷ Furthermore, it has been reported that the effect of gabapentin persists in the presence of the GABA (B) antagonist, saclofen.⁴⁸

Na⁺ channels. It has been shown that neither the binding of [³H]-batrachotoxin to rat brain membranes⁴⁹ nor the Na⁺ currents in transfected ovary⁵⁰ nor the Ca²⁺ influx into brain synaptosomes induced by veratridine, are affected by GBPs.⁵¹ After acute exposure to these compounds, no effect was observed on the repetitive firing of Na⁺ dependent action potentials of cultured spinal cord and neocortical neurons.⁵²⁻⁵⁴ GBPs were found to have no effect on neuronal Na channels in a variety of neurons.^{42,55} However, in neuropathic rats these drugs suppressed ectopic discharges from injured sciatic nerves and decreased the number of action potentials during depolarization in injured dorsal root ganglion (DRG) neurons.^{53,54,56} These results suggest that GBPs might partly exert its analgesic effect by affecting the Na⁺ channels of injured nerves. This difference in the efficiency of GBPs might be related to changes of Na⁺ channels type expression associated to nerve injury.⁵⁷

K⁺ channels. Controversial data have been reported on the effect of GBP on ATP sensitive potassium channels.^{58,59} An enhancement of voltage activated potassium current in DRG neurons via protein kinase A was suggested.⁶⁰ In addition to the effect on potassium channels GBP has also been shown to modulate the hyperpolarization-activated cation current (I_h).⁶¹ Surges⁶¹ reported that gabapentin increased I_h in rat CA1 pyramidal cells through a cAMP-independent mechanism resulting in a several millivolts depolarization of membrane potential. This effect was proposed to contribute to the antiepileptic effect of GBP by decreasing the sensitivity to excitatory inputs.⁶² However, Cheng⁶³ published that gabapentin did not produce any depolarization in ventrolateral neurons of rat periaqueductal gray slices.

Acute Modulation of Ca²⁺ Channels

Ca²⁺ channel $\alpha_2\delta$ -1 and $\alpha_2\delta$ -2 subunits were proposed to be the main site of action of GBPs.⁶⁴ Since individual $\alpha_2\delta$ subtype may associate (or form complexes) with different α_1 VGCC,²³ it is expected that GBPs should affect various types of Ca²⁺ channels depending on the tissue and/or cell studied. In fact the expression of $\alpha_2\delta$ subunit mRNA containing cells mapped by in situ hybridization method shows that specific labeling of the different $\alpha_2\delta$ subunits type was widely, although differentially, distributed in neurons in the brain, the spinal cord and the DRG.⁶⁵ Therefore it is not surprising that gabapentin effect is not similar in all cell preparations a finding which may reflect a biovariability in the target binding interactions of GBPs.

Several reports indicate that GBPs reduced the cellular influx of Ca²⁺ via VGCC in synaptosome fractions prepared from brain tissue.⁶⁶⁻⁶⁸ GBP application results in acute inhibition of Ca²⁺ currents, particularly L-type Ca²⁺.^{69,70} However, in most studies, acute inhibition by GBPs is either minor or absent.¹⁸ Similarly studies with recombinant VGCC have failed to show any acute effects of gabapentin on channel function. In fact Kang⁷¹ showed, through neuronal recombinant P/Q-type Ca²⁺

channels expressed in *Xenopus* oocytes, that Ba²⁺ currents are not affected by acute treatment with 30 μ M of gabapentin, although it slows down the kinetics of inactivation in a dose-dependent manner after chronic exposure to the drug.⁷¹ The failure to shown an effect on these calcium currents could be caused by the fact that recombinant channels lack several interacting proteins (e.g., syntaxin, synaptotagmin and β subunits), which are found at synapses. Alternatively, the use of Ba²⁺ instead of Ca²⁺ as an ion carrier could have masked the effect of the GBPs on a Ca²⁺-dependent inactivation.

Electrophysiological recordings have failed to detect any acute gabapentin-mediated change in Ca²⁺ channel currents recorded from hippocampal neurons taken from patients with temporal lobe epilepsy.⁷² Also, there was no effect of GBPs on Ba²⁺ currents from Purkinje cells, despite the fact that these cells are known to express $\alpha_2\delta$ -2 as their predominant $\alpha_2\delta$ subunit.^{18,23} Similarly, negligible inhibition of Ca²⁺ channel activity was observed in PC12 cells after acute treatment, but a significant decrease in Ca²⁺ current amplitude was promoted by chronic exposure to GBPs.⁷³

On the other hand, there are many reports with direct and indirect evidence showing that GBPs do affect Ca²⁺. Acute application of GBPs has been demonstrated to inhibit L-type Ca²⁺ currents in isolated rat neurons,⁶⁹ but these studies have not yet been confirmed on rat dentate gyrus,⁷⁴ mouse skeletal myotubes,⁷⁵ and neuronal N-type currents of cultured rat dorsal root ganglion neurons.⁴⁷

It was reported that the sensitivity of Ca²⁺ currents to gabapentin is greatly influenced by the relative amount of $\alpha_2\delta$ messenger RNA (mRNA) present in cells, presumably as a result of an increased incorporation of specific subunits in the expressed Ca²⁺ channel complexes.⁷⁰ The physiological state of the cells also influences the effects of GBPs on Ca²⁺ currents from DRG cells. GBPs significantly reduced whole cell Ca²⁺ current amplitude at positive membrane potentials when a pulse preceded the test pulses or when cells were stimulated with a train of pulses. In control cells, neither pre-pulse depolarization nor pulse trains reduced Ca²⁺ currents at positive potentials.⁷⁵ GBPs, administered at clinically relevant concentrations, resulted in significant reduction of Ca²⁺ currents in both sham and neuropathic DRG primary afferent neurons, while in non-operated rats reduced Ca²⁺ current to a smaller degree.⁵⁸ Some of these differences among different studies may be explained by the changes in the expression and sensitivity to pharmacological blockades observed in genetically modified mouse that overexpress $\alpha_2\delta$ type-1.⁷⁶

Acute Modulation of Neurotransmitter Release

Despite the puzzling effects of GBPs on Ca²⁺ currents most of the studies where the effect of these compounds on transmitter release were analyzed showed a reduced release of various neurotransmitters from synapses in several neuronal tissues.³²

Pregabalin has been previously used in a broad range of concentrations (0.25 μ M to 1 mM^{34,60,77}). Pharmacokinetics showed that single in-vivo pregabalin dose administration to patients reached clinical plasma concentrations to 120 μ M,⁷⁸

while higher concentrations such as 500 μM were expected after multiple doses (plasma half-life ~ 6 hours⁷⁷). Furthermore, gabapentin was suggested to be 4- to 8-fold more concentrated in the brain than in blood plasma.^{2,79} Finally, different effective concentrations of GBPs might be observed in different synaptic preparations (hippocampus, trigeminal nucleus, neuromuscular junctions, etc.) according to their differential interaction with $\alpha_2\delta$ auxiliary subunit and synaptic proteins. Both, P/Q type ($\text{Ca}_v2.1$) and N-type ($\text{Ca}_v2.2$) channels are implicated in transmitter release early during development. However, in more mature cells, the P/Q-type Ca^{2+} channels become predominant, as shown at peripheral and central nervous system synapses.⁸⁰⁻⁸³ P/Q type channels have been implicated in the effect of GBPs on K^+ -evoked release of noradrenaline and glutamate in rodent spinal cord and cortical slices.^{33,48,84} N-type channels mediating excitatory postsynaptic potentials in rat hippocampal slices are sensitive to GBPs.⁸⁵ By contrast, L-type channels, not typically involved in neurotransmission, are not sensitive to GBPs even in conditions where they mediate K^+ -evoked [^3H]-noradrenaline release by L-type activators.⁴⁸ Whether $\alpha_2\delta$ ligands differentially target these VGCC to modulate the release process requires further investigation.

Neurochemical experiments provided the first evidence to suggest that GBPs can alter neurotransmitter release by measuring electrically evoked, Ca^{2+} -dependent [^3H]-monoamine release from rat neocortical slices or rabbit striatal slices.^{86,87} More recently GBPs were tested on veratridine-, electrical- and K^+ -evoked stimulation using rat neocortical slices pre-labeled with [^3H]-norepinephrine. The inhibition by these drugs was most pronounced with the K^+ stimulus and absent with veratridine-evoked release.⁴⁸

The GBP effect on K^+ -evoked release of pre-labeled neurotransmitters was also studied in human neocortical slices. A significant inhibition of K^+ -evoked [^3H]-acetylcholine, [^3H]-noradrenaline and [^3H]-serotonin release (between 22% and 56%) was observed without affecting [^3H]-Dopamine release. The GBPs were ineffective in the presence of the putative $\alpha_2\delta$ antagonist L-isoleucine but not with the D-enantiomer.³⁶

Capsaicin-evoked substance P and calcitonin gene-related peptide (CGRP) release from rat spinal cord slices were also not altered by GBPs in non-inflamed animals.⁸⁸ Gabapentin was shown to increase the ratio between background synaptic inhibition to excitation and to decrease neuronal excitability in neurons of the rat entorhinal cortex in vitro.⁸⁹ In agreement with a small effect of GBPs, K^+ -evoked glutamate release from rat neocortical and hippocampal slices has been reported (11–26%).⁹⁰ However, GBPs had no effect on exocytotic [^3H]-glutamate release from human neocortical synaptosomes but were capable of substantially reduced K^+ -evoked [^3H]-GABA release.³⁶ Thus these experiments do not support the hypothesis that the anticonvulsant action of GBPs is exerted via inhibition of glutamate release.

In contrast, electrophysiological studies using gabapentin and pregabalin substantiated reductions in glutamate and glycine release in neocortical, hippocampal and spinal cord slices.^{33,68,84,85} Furthermore, several reports showed that asynchronous release which occurs without presynaptic action potentials is reduced by

GBPs suggesting that these drugs might affect a Ca^{2+} independent release mechanism.^{33,34,91}

Pregabalin Modulation of Neuromuscular Transmitter Release: A Model of Synaptic Transmission Mediated by P/Q-type Calcium Channels

The $\alpha_2\delta$ -1 subunit is the neuronal Thrombospondin (TSP) receptor that is required for central nervous system synapse formation.⁹² Muscle cells are rich in Thrombospondin-4 (TSP-4)⁹³ and $\alpha_2\delta$ -1.⁹ Both TSP-4 and $\alpha_2\delta$ -1 proteins appear to accumulate in the postsynaptic area of neuromuscular junctions (NMJ) or muscle, respectively. There is an absence of detectable levels of TSP-4 mRNA in spinal motoneurons⁹³ and only mRNA $\alpha_2\delta$ -3 is densely expressed in the cell bodies located at the ventral horn of spinal cord.⁶⁵ Since, the proteins $\alpha_2\delta$ -3 and 4 are known to not bind gabapentin,^{23,94} the role of TSP-4- $\alpha_2\delta$ interactions is unclear. In addition, an acute gabapentin dose of 300 μM did not affect basic electrophysiological parameters at either adult wild-type or ducky (*du*) neuromuscular transmission.⁹⁵

According to these results, adult motor nerve terminals should be insensitive to gabapentinoids. However, preliminary data from our group showed clear effects of pregabalin, affecting presynaptic parameters during neuromuscular transmission. We used intracellular recordings to study neuromuscular transmission, as previously described in reference 80 and 96. The miniature Endplate Potentials (mEPPs) did not show any change in amplitude after acute bath application of 1 mM pregabalin. Mean mEPPs frequency was significantly increased (2 ± 0.5 and 4.3 ± 1.2 Hz, in control and pregabalin conditions, respectively; Student's-t-test, $p < 0.05$, $n > 7$ NMJ). Thus, suggesting that pregabalin did have a presynaptic, but not postsynaptic action at mice neuromuscular junctions. Further indications of a presynaptic pregabalin action were obtained during nerve stimulation at high frequency (100 Hz, 30 stimuli). Indeed, the 100 Hz-induced depression of endplate potentials that normally follows a single exponential decaying time course, was changed by pregabalin (500 μM) to a biphasic facilitation-depression time course. Moreover, both increment in spontaneous mEPPs frequencies and initial facilitation during 100 Hz trains of nerve stimulation suggest a pregabalin-mediated effect on calcium channels influx at presynaptic neuromuscular terminals.

Pregabalin Modulation of Neurotransmitter Release and P/Q-type Calcium Channels at the Central Nervous System Synapse

In order to determine the action of pregabalin in a preparation where presynaptic Ca^{2+} currents as well as transmitter release could be investigated in detail we studied the effect of pregabalin on the calyx of Held-Medial Nucleus of the Trapezoid Body (MNTB) synapse. As we shown at the NMJ, at the calyx—MNTB synapse the amplitude of the miniature excitatory postsynaptic currents (EPSCs) were not affected while the amplitudes of EPSCs were reduced in a dose dependent manner with a maximal effect of 30% inhibition at 500 μM of pregabalin.

We studied the presynaptic action of pregabalin on Ca_v2.1 (P/Q-type) calcium channels under whole cell voltage clamp conditions at the presynaptic calyx of Held³⁸ nerve terminal. A clinical high-concentration dose of pregabalin (e.g., 500 μM) blocked Ca_v2.1 channel-mediated currents evoked by both depolarizing voltage square pulses and ramp protocols, without changing their voltage-dependent activation. Most interestingly, pregabalin at both 100 μM and after 500 μM was able to reduce inactivation of presynaptic calcium, but not barium-mediated presynaptic currents. On the other hand, pregabalin increased the activation speed of Ca²⁺ currents. These data together with the lower inactivation observed in Ca_v2.1 channels might justify the observed decrease of presynaptic short-term facilitation. Our ongoing results suggest that pregabalin affects presynaptic Ca²⁺ currents in three ways: (1) by blocking presynaptic P/Q-type mediated calcium currents, thus reducing synaptic transmission, (2) by increasing activation speed which will counteract a substantial reduction of the action potential elicited calcium influx and 3) by reducing the inactivation of P/Q channels, which would allow a fast recovery of Ca²⁺ currents during high frequency synaptic stimulation.

Concluding Remarks

The direct action of GBPs on VDCCs appears to be essential for their modulatory effects on the release of multiple types of neurotransmitters. Moreover, such modulation is highly dependent upon the interaction between these Ca²⁺ channels and their

environment, the state of the Ca²⁺ channels and the levels of expression and type of their auxiliary subunits.

Drastic changes on neuronal environment appeared to have an impact on GBP effects. Indeed, PGB-mediated alterations were enhanced by conditions like prolonged depolarization, inflammation, hyperexcitability or activation of protein kinase C and adenylylase.^{48,88,91,97,98}

Thus, the apparent lack of acute effects of GBPs might be easily challenged by characterizing their effects on animal models of different diseases. Synaptic transmission under pathological states of α₂δ subunits expression should be more reactive to GBPs than under baseline, control conditions.

In conclusion, future directions to understand GBPs effects should consider the existence of a novel mechanism whereby GBPs have a less effect on physiological transmitter release but significantly affect 'sensitized' or 'abnormal' neurotransmitter release.

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