Review

GABA Receptor Subtypes Involvement of Analgesia: Current Status

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Abstract: Gamma-aminobutyric acid (GABA), a non-protein-producing amino acid, is extensively found in microorganisms, plants and vertebrates, and is abundantly expressed in the spinal cord and brain. To date, GABA is considered to be the major inhibitory neurotransmitter in the central nervous system. Its physiological effects are related to the regulation of synaptic transmission, the promotion of neuronal development and relaxation, and the prevention of insomnia and depression. Mediated through its specific receptors, it plays a pivotal role in the control of neuronal excitability, which can serve as anovel target for developing analgesics for pain management. This review provides an update on the accumulating evidence of specific GABA receptors and their subtypes in the involvement of pain analgesia.

Keywords: Gamma-aminobutyric acid; subtype; analgesic effect; mechanism

1. Introduction

Chronic pain impacts more people than heart disease, cancer, and diabetes combined[1]. Chronic pain includes many different forms with different causes and neuropathic pain is one of the most neurological disorders owing to the deficiency of effective clinical treatment[2]. Neuropathic pain refers to a chronic pain induced by an injury or disease regarding the peripheral or central nervous system[3].It affects 7-10% of the global population and has a serious influence on the life quality of patients[4].The condition is under-recognized and under-diagnosed, and its clinical management is a real challenge for physicians. The currently available treatment options focus on clinical symptoms, which usually have limited efficacy[5,6] and are often related to adverse side effects[4,7].

Neuronal synapses mediate the pain signal transduction from presynaptic neurons to postsynaptic neurons through the synaptic gap. Neuronal activity is heavily dependent on the dynamic equilibrium between inhibitory and excitatory functions[8]. The firing patterns of neuron in the brain are strongly regulated by the inhibitory neurotransmitter gamma-aminobutyric acid (GABA)[9].

GABA is the main inhibitory neurotransmitter existed in the central nervous system (CNS) and acts through two different types of receptors, the ionotropic $GABA_A$ and $GABA_C$ receptors and the metabotropic $GABA_B$ receptors[10]. In general, GABA is synthesized in presynaptic nerve cells from L-glutamate, stored in synaptic vesicles, and then catalyzed by glutamic acid decarboxylase. GABA is released into the synapse when the presynaptic neuron receives a pain signal, i.e. when the neuron is excited. GABA is important in human health, where it is involved in a variety of physiological roles and has a wide range of applications in pharmaceutical and food industries.

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2. The role of GABA and its receptors in pain transduction and modulation

As stated above, there are three types of GABA receptors: alpha or A, beta or B, and gamma or C, which recognize and bind GABA; these receptors are primarily located on the postsynaptic membranes. $GABA_A$ and $GABA_C$ receptors belong to ligand-gated ion channels, while $GABA_B$ receptors pertain to G protein-coupled receptors. $GABA_A$ receptors mediate fast synaptic transmission, while $GABA_B$ receptors mediate slow synaptic transmission. Evidence shows that $GABA_A$ receptors are involved in epilepsy, resistance to injury, anxiety, and panic, while $GABA_B$ receptors are involved in memory, mood and pain modulation. Although $GABA_C$ receptors have been identified, their physiological function is not yet fully understood[11].

2.1. The role of GABA in pain transduction and regulation

GABA is widely distributed in the neuraxis and, according to previous studies, plays a vital role in regulating most central nervous system functions due to its widespread presence and relatively high levels in the spinal cord and brain. The agonists and antagonists of GABA receptor exhibit an extensive range of pharmacological activities including anxiolysis, hypnosis, amnesia, muscle relaxation, cognitive enhancement, euphoria and anticonvulsant activity[12].

As a neurotransmitter, GABA is released by one neuron to transmit information to another neuron. It is stored in membrane vesicles at the end of axons. There are thousands of GABA molecules stored in each vesicle. The vesicles are fused to the nerve cell membrane and the GABA molecules are released by the neuron and then by the cytosol. The GABA molecules subsequently are released into the synaptic gap and diffuse through the synaptic gap to the postsynaptic neuron. In humans, GABA functions at inhibitory synapses via binding to GABA receptors, which leads to the opening of ion channels, thus allowing potassium ions to flow out of the cell and chloride ions to flow in[13]. This action leads to a negative variation of transmembrane potential, which causes hyperpolarization and reduces neuronal excitability, presenting an analgesic effect. The released GABA in the synaptic gap that is not bound to the receptor is either degraded by enzymes or returned to the presynaptic axon terminal via active transport pattern by transporters or reuptake pumps[11].

An increase of GABA levels has been found to be correlated with the nocifensive behaviors in formalin test, suggesting the important role of GABA in pain sensations[14]. This probably is not surprising as it has long been proposed in the gate control theory of pain that the GABAergic neurotransmission in the dorsal horn of spinal cord plays a crucial role in controlling the transmission of pain signals. Braz et al[15] provided further evidence in supporting this theory by intraspinal transplantation of cortical precursors of GABAergic interneurons from the embryonic medial ganglionic eminence (MGE) to restore spinal cord GABAergic signaling. They demonstrated that MGE cell transplants reversed the mechanical allodynia and thermal hyperalgesia in nerve injury- and chemotherapy (paclitaxel)-induced models of neuropathic pain in mice. In contrast, the transplantation of cells with a deletion of the vesicular GABA transporter, which is required for the storage of synthesized GABA, has no effect on the pain-like behaviors, indicating that GABA released from these cells are crucial for the transmission of pain sensation[15,16]. These studies demonstrate that GABA plays important roles in pain transduction and regulation.

2.2. The role of $GABA_A$ receptors in pain transduction and modulation

The relationship between $GABA_A$ and analgesia has been becoming a hot pursuit in pain research in recent years. $GABA_A$ receptors are the most important of the three subtypes of receptors and are currently the most intensively studied. $GABA_A$ receptors belong to pentameric ligand-gated ion channels mediating most of the rapid inhibitory synaptic transmission in central nervous system, and $GABA_A$ receptor dysfunction is associated with many psychiatric and neurological disorders, such as anxiety, epilepsy and substance use disorders[9]. The $GABA_A$ receptor is comprised of five distinct subunits whose expression varies according to brain region, cell type and subcellular structural domain, as well as their function. To date, at least 19 receptor subunits are found, which are divided into subclasses according to homologous amino acid sequences: α1, α2, α3, α4, α5, α6, β1, β2, β3, and γ1, γ2, γ3, δ, ε, π, θ, and ϱ1, ϱ2, ϱ3[17]. Although the receptor subunits vary in

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combination and function, not all subunits assemble efficiently into functional receptor subtypes. Most $GABA_A$ receptors include two α-, two β- and one γ- subunits[18,19], which form an integrated negative ion channel that is permeable to chloride and bicarbonate ions. $GABA_A$ receptors are primarily involved in antinociceptive effects at the supraspinal level, modulating GABA-induced inhibition of short duration monosynapses. When GABA combines with $GABA_A$ receptors, chloride ion permeability on the nerve membrane increases, causing an anion-selective channel to open. This channel mainly gates chloride ions which allows them to flow inside neurons via ion concentration gradients, leading to hyperpolarization of the cell membrane, thereby inhibiting the neural excitability of GABAergic interneurons. As a result, this reduces pain signal transduction and further produces analgesic effect (see Figure 1)[20].

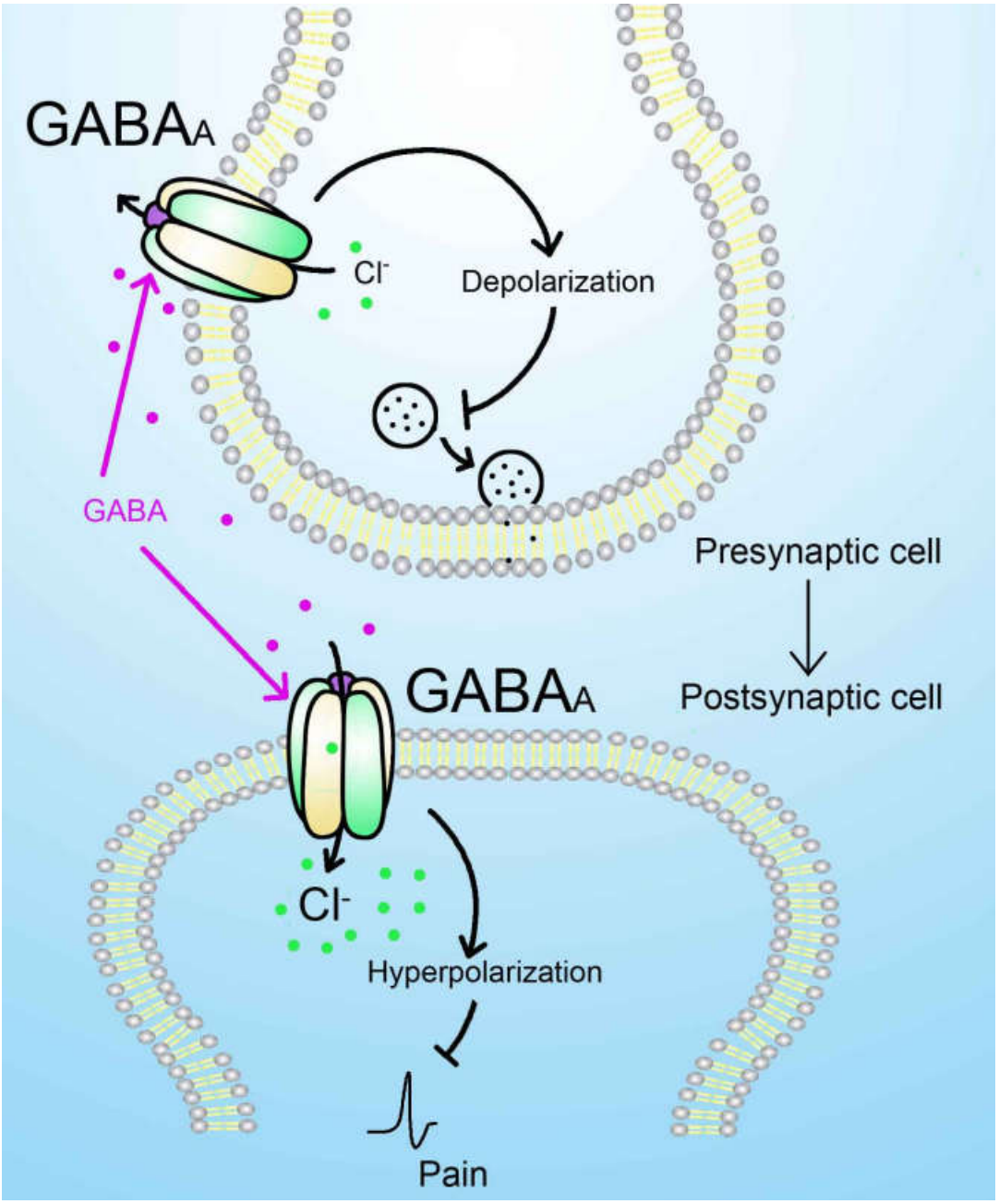

Figure 1. $GABA_A$ receptor-mediated analgesic effect. Presynaptic $GABA_A$ receptors activation leads to Cl^- efflux because of the high concentration of Cl^- in the intracellular side of primary afferent neurons. The initial depolarization reduced the neurotransmitter release induced by noxious stimuli and pain signaling generation. Postsynaptic $GABA_A$ receptor activation leads to Cl^- influx because of low concentration of intracellular Cl^-, which hyperpolarizes the secondary sensory neurons and lowers their excitability reducing the paining sensation.

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GABA$_A$ receptors are the most prevalent receptors of inhibitory neurotransmitter in the CNS, and primary afferent neurons (PAD) are principally depolarized by GABA through the action of the cationic chloride transporter protein (NKCC1)[21]. NKCC1 helps maintain a high intracellular concentration of chloride ion (Cl^-)[22], and the binding of GABA to GABA$_A$ receptors leads to anion efflux to depolarize PAD. The subsequent shunting of afferent action potentials leads to a decrease in excitatory transmitters released from injurious terminals. This process is known as presynaptic inhibition and plays a crucial role in controlling the hyperexcitability of neurons in the dorsal horn of the spinal cord[23]. However, there are exceptions. For example, certain types of injury reportedly can enhance PAD such that spiking potentials can be sequentially evoked at primary afferent terminals, thus leading to the change in the PAD from a normal inhibitory process to an excitatory one[24].

2.2.1. Mechanism of analgesic action of alpha(α) subtype receptors

Studies have shown that the cerebral cortex, hippocampus, and caudate nucleus exhibit complex expression patterns of multiple GABA$_A$ receptor subtype combinations. Subunits are expressed in the hypothalamus, striatal-nigral fibers and cerebellar stellate/basket cells, and are also extensively existed in the spinal cord. Alpha (α) subunits are important determining factors for localization and function of receptors. The two main isoforms of α, α1 and α2, are widely distributed in the brain. Especially, the most abundant subunit, α1, is almost ubiquitously present in the brain[25]. The α1 subunit in the spinal cord is concentrated around the central canal. Although the α2 subunit is widely expressed, the regional expression patterns of the α1 and α2 subunits in the brain are negatively correlated. The α2 subunit is highly present in the cerebellum and forebrain, including the amygdala, hippocampus, olfactory bulb, lateral septum and granule cells of the medial preoptic area of the hypothalamus, and is most abundant in the superficial dorsal horn of the spinal cord. The α3 subunit expression is localized in the olfactory bulb, cerebral cortex and brainstem nuclei, and is also distributed throughout the dorsal horn of the spinal cord and around the central canal. The α4 subunit expression is limited to the hippocampus, thalamus and striatum. The α5 subunit is abundant in the hippocampus and is also expressed in the granule cells of the olfactory bulb, neocortex and hypothalamus, but is less abundant[26]. It is also weakly expressed in the spinal cord. The expression pattern of the α6 subunit is the most restricted of the α subunits, existing in cochlear nucleus granule cells and cerebellar granule cells[27]. Taken together, α1, α2 and α3 are mainly concentrated at synaptic sites, whereas α4, α5 and α6 are usually located extrasynaptically[28].

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Table 1. Main distribution of the alpha subunit of the GABA$_A$ receptor

Subunit	Main distribution areas
α_1	Hippocampus, cerebral cortex, pericentral canal of spinal cord
α_2	Cerebellum, forebrain, superficial dorsal horn of spinal cord
α_3	Cortical, dorsal horn of the spinal cord and pericentral canal
α_4	Striatum, thalamus
α_5	Olfactory bulb, hippocampus
α_6	Cochlear nucleus granule cells, Cerebellar granule cells

The involvement of the GABA$_A$ receptor subunit in analgesia has been well documented. Among the GABA$_A$ receptor binding sites, we focus on benzodiazepine receptors, which are often used in chronic pain patients to facilitate their sleeping. However, benzodiazepines do not have clear analgesic efficacy, particularly when they are administered systemically. The high affinity of the benzodiazepine receptor binding site needs the existence of a histidine residue at a conserved site in the *N*-terminal structural domain of the α subunit, which is present in the α1, α2, α3, α5 subunits but not in the α4 and α6 subunits. Mutation of histidine residues to arginine effectively reduces the affinity of GABA$_A$ receptors for benzodiazepine receptor binding sites without altering their response to GABA. McKernan *et al.*[29] reported that the sedative effect of benzodiazepines is primarily mediated through the α1-GABA$_A$ receptor as a significant reduction in the sedative effect of benzodiazepines was observed in mice carrying the α1 subunit H→R point mutation. In contrast, one study by Crestani *et al.*[30] found that point mutations in the α2 subunit could lead to a loss of anxiolytic effects of benzodiazepines. Through the study of benzodiazepine-mediated effects in mice with various α-subunit point mutations[31], it is generally believed that GABA$_A$ receptors containing α1 subtype mediate sedative effects, whereas receptors containing α2/α3 are associated with anxiety and those containing α5 are associated with memory function.

Knockout mice proves to be a powerful tool to dissect the specific involvement of different α subtypes in pain sensation. In a series of studies, specific point mutant mice were created, i.e. knocking out one of four alpha subtype: α1H→R(RHHH), α2H→R(HRHH), α3H→R(HHRH), α5H→R(HHHR)[32]. Mouse models of inflammatory pain and neuropathic pain were then established by injecting formalin into the hind paw or performing chronic constriction injury surgery (CCI). After intrathecal injection of diazepam into these mice, respectively, the antinociceptive/hyperalgesic effect was found to be significantly reduced in α2 (HRHH) and α3 (HHRH) point-mutant mice, suggesting that GABA$_A$ receptors containing α2 and α3 subtypes in the spinal cord mediated the analgesic effect of intrathecal diazepam administration and were the main contributors[32]. In addition, a minimal effect of spinal GABA$_A$ receptors containing α5 was found in the α5 (HHHR) point-mutant mice in a model of inflammatory pain. Compound NS11394 is a partial agonist with functional selectivity for α5>α3>α2>α1 GABA$_A$ receptors[33]. In rodent central sensitization-related pain models, NS11394 can increase mechanical pain and thermal pain thresholds and effectively alleviate arousal behavior in rats. After treated with flumazenil, the effect of NS11394 was reversed, thus demonstrating that NS11394 can produce antinociceptive effects by binding to GABA$_A$ receptors *in vivo*[34]. NS11394 was then made a comparison with other known positive allosteric modulators. Knabl *et al.*[32] selected an anxiolytic and selective GABA$_A$ receptor partial agonist, L-838417, which positively modulates α2, α3 and α5 GABA$_A$ receptors (43%, 43%, and 39%), but lacks α1 GABA$_A$ receptor activity (1.5%)[35]. L-838417 not only is an α2, α3 and α5 partial agonist, but also is an α1 antagonist. Since α2, α3 and α5 subunits are the major α subunits in the spinal cord, and α1 subunits are mainly expressed in the periaqueductal gray (PAG). L-838417 may positively modulate spinal antinociceptive alloreceptors while blocking central prenociceptive GABA$_A$ receptors. Both actions contribute to its antinociceptive effects, suggesting that L-838417 has analgesic effects in inflammatory and neuropathic pain models in wild-type rats. These findings

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suggest that α2/α3 selective or α2/α3/α5 selective agonists may be a new class of analgesics that can be used alone or in combination with existing analgesics for disorders associated with inflammatory pain or neuropathic pain. This demonstrates that, in addition to selectivity *per se*, a minimal level of positive regulation of individual α subunits (α2, α3 and possibly α5) may be a key determinant of antinociceptive hyperalgesia. On the other hand, TPA023 also activates α2 and α3 $GABA_A$ receptors. Although TPA023 is not as potent as L-838417, it also lacks activity at α1 $GABA_A$ receptors and is significantly less potent at α5 $GABA_A$ receptors[36]. It is an α2/α3 selective partial agonist, but the analgesic effect is not significant, probably due to insufficient α2/α3 efficacy[37]. Similarly, compound HZ166, an imidazolobenzodiazepine with selective partial agonism for receptors containing α2 and α3 subunits, showed reversible hyperalgesic effects of flumazenil in mice with yeast polysaccharide A-induced inflammation and CCI-induced mechanical pain hypersensitivity[38], which demonstrated its analgesic effects in mouse models of neuropathic and inflammatory pain. Taken together, NS11394, L-838417, and HZ166 are all potent agonists (positive allosteric modulators) at α2/α3-$GABA_A$ receptors with *in vivo* analgesic efficacies in preclinical studies.

Ralvenius *et al.*[39] also bred four point mutant mice with only one $GABA_A$ receptor subtype remaining benzodiazepine-sensitive, HRRR, RHRR, RRHR, RRRH (mice only with α1 or α2 or α3 or α5-GABAA receptors are sensitive to benzodiazepines), and tested them with diazepam. Although diazepam is a classic non-selective benzodiazepine, its activity was only restricted to a single $GABA_A$ subtype in these triple point mutant mice. The results showed that the order of action of the subtypes was α2>α5>α3 for anti-mechanical hyperalgesia, and α2>α3>α5 for anti-thermal hyperalgesia and chemical injury. These results further support the critical role of α2-$GABA_A$ receptor as an antihyperalgesic target. The lack of consistent analgesic effect of benzodiazepines such as diazepam is likely due to their broad pharmacological actions which overshadow the *bona fide* analgesic efficacy. These studies facilitate the enthusiasm of developing subtype-selective (i.e., α2 and α3-specific) benzodiazepines for pain management.

2.2.2. Mechanism of analgesic action of delta(δ) subtype receptors

$GABA_A$ receptors can be divided into synaptic receptors and extrasynaptic receptors based on their distribution within neurons. The two receptor groups also have distinct molecular structural features: synaptic receptors typically contain the γ subtype, while most extrasynaptic receptors are composed of the delta (δ) subtype. Unlike the α subtype $GABA_A$ receptors which are regulated by benzodiazepines through the benzodiazepine receptor binding site, the extrasynaptic delta-subtype-containing $GABA_A$ receptors are insensitive to positive allosteric modulation by benzodiazepines[40]. Instead, they are highly sensitive to the modulation of neurosteroids.

Peng *et al.*[41] demonstrated the protein expression of δ-$GABA_A$ receptors in the isolated spinal cord by Western blotting. In another study[42], the presence of δ-$GABA_A$ receptor mRNA was found by reverse transcription-polymerase chain reaction analysis of lamina tissue. These results suggest that δ-$GABA_A$ receptors are present in the spinal cord, By electrophysiological recording, neurons in the spinal cord laminae were found to display a δ-$GABA_A$ receptor-mediated tonic inhibitory current[43]. One known δ-$GABA_A$ receptor-preferring agonist with analgesic properties is THIP. Studies showd that THIP's enhancement of δ-$GABA_A$ receptor activity reduced the excitability of spinal neurons in wild-type mice, but not in $Gabrd^{-/-}$ mice (δ-$GABA_A$ receptor-deficient mice). Compared to GABA, THIP excites greater currents from $GABA_A$ receptors containing the δ subunit than from $GABA_A$ receptors lacking the δ subunit[44]. The δ-$GABA_A$ receptor produced low-amplitude tonic inhibitory currents on spinal lamina II neurons, suggesting its potential as a pharmacological target for reducing acute injurious sensations by modulating central sensitization[45]. Another compound with well-characterized analgesic efficacy was flupirtine, whose analgesic activity has been demonstrated in various animal models and in humans[46]. Flupirtine has long been thought of as a selective neuronal potassium (K^+) channel opener[47]. However, Klinger *et al.*[48] found that flupirtine has different effects on synaptic and extrasynaptic $GABA_A$ receptors and preferentially acts on extrasynaptic $GABA_A$ receptors. This suggests that δ-

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containing $GABA_A$ receptors may be an important target of flupirtine and its analgesic activities may be at least partially mediated through δ-containing $GABA_A$ receptors. In summary, there are some evidence in the literature suggesting that δ-$GABA_A$ receptors may also be involved in pain modulation and warrants further examination.

2.3. The role of $GABA_B$ receptors in pain transduction and modulation

$GABA_B$ receptors, first identified by Dr. Norman Bowery in 1979, belong to the G protein-coupled receptor superfamily with seven transmembrane segments. The functional $GABA_B$ receptor is a heterodimer composed of $GABA_{B1}$ and $GABA_{B2}$ subunits. $GABA_{B1}$ subunits possesses the ligand-binding site mediating the interaction between the receptor and the ligand, while $GABA_{B2}$ subunit mediates the downstream signaling via associated G proteins and modulates the affinity of $GABA_{B1}$ subunit to GABA. Both $GABA_{B1}$ subunit and $GABA_{B2}$ subunit have an intracellular C-terminal domain, an extracellular N-terminal domain, and a core transmembrane domain. $GABA_{B1}$ subunit has several splice variants with $GABA_{B1a}$ and $GABA_{B1b}$ as two most common varaints. The presence of two sushi domains on the $GABA_{B1a}$ subunit makes it distinct from the $GABA_{B1b}$ subunit, which affects the transportation of the receptor[49]. Upon activation, $GABA_B$ receptor mediates slow and prolonged inhibitory effect via the activation of the associated $G_{i/o}$-protein. On the pre-synaptic side, the dissociated $G\alpha i$ subunit usually inhibits the activity of adenylyl cyclase and reduces the production of cAMP, leading to the reduced neurotransmitter release, while the $G_{\beta\gamma}$ segment can inhibit the voltage-gated calcium channel, decreasing the vesicle fusion and transmitter release. On the post-synaptic side, $GABA_B$ receptor activates the inwardly rectifying potassium channels, hyperpolarizing the membrane potential, decreasing the excitability. The inhibitory effects of $GABA_B$ receptor make it a promising therapeutic target in pain management[50]. The schematic diagram for $GABA_B$ receptor-mediated analgesic effect are shown in Figure 2.

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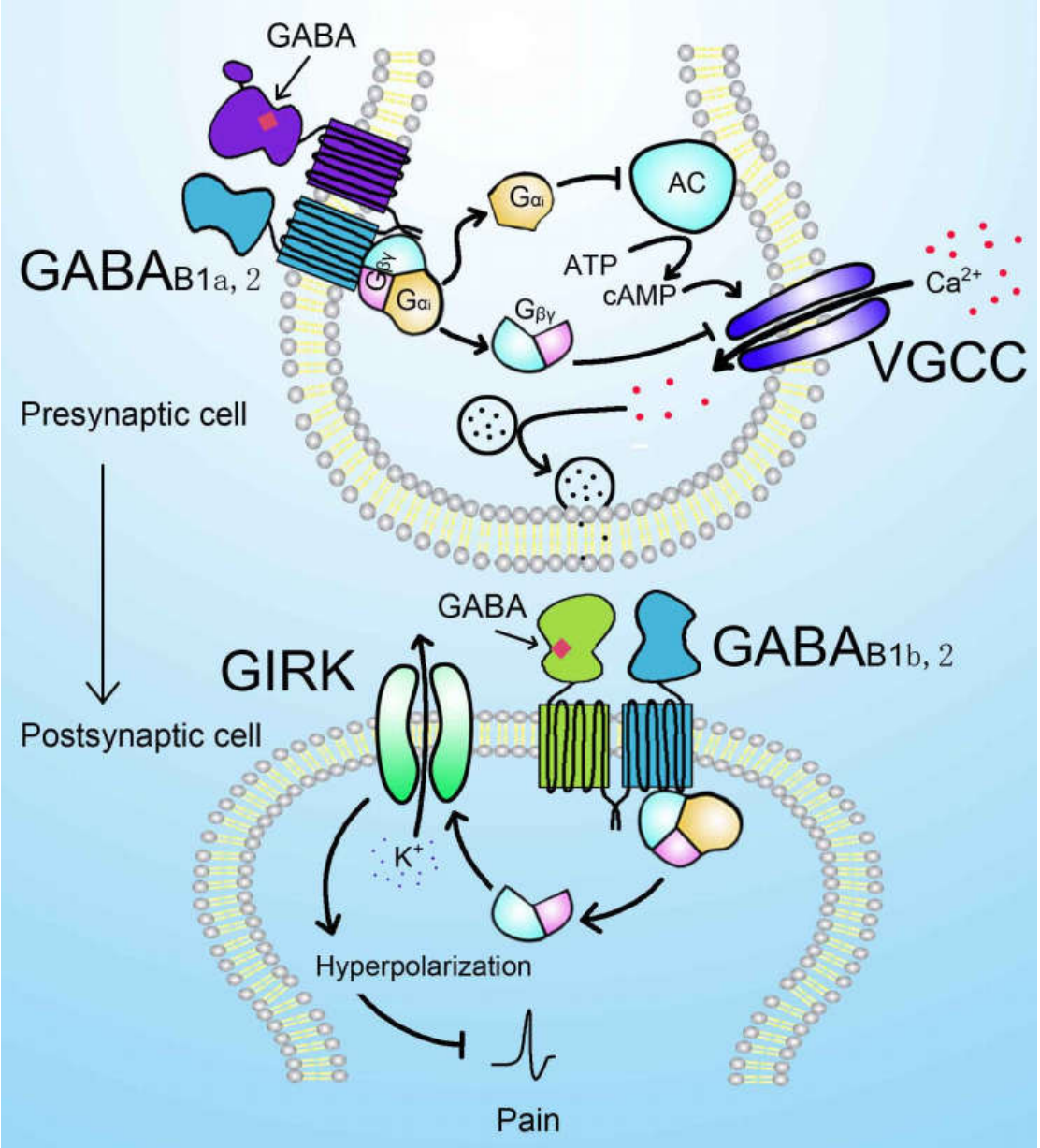

Figure 2. $GABA_B$ receptor-mediated analgesic effect. Presynaptic B1a-containing $GABA_B$ receptor interaction with GABA promotes the activation of G_i protein. The $G\alpha_i$ inhibits the activity of adenylyl cyclase (AC) reducing the production of cAMP, which enhances the function of voltage-gated calcium channel (VGCC). The $G\beta\gamma$ can directly inhibit the function of VGCC. Both pathways contribute to the reduced activity of VGCC and neurotransmitter release. The postsynaptic B1b-containing $GABA_B$ receptor activation leads to the activation of G protein-gated inwardly rectifying potassium (GIRK) channels via $G\beta\gamma$ complex. GIRK channel opening promotes the efflux of K^+ and hyperpolarization, which reduces the excitability of the postsynaptic cell and exerts analgesic effect.

$GABA_B$ receptors are widely expressed in the central and peripheral nervous system exhibiting a development- and region-specific manner, including the thalamus, brainstem nuclei, and spinal cord and play a variety of important roles, such as synaptic development and memory[51]. The extensive expression of $GABA_B$ receptors in the pain sensing neurons in the superficial layers of the dorsal horn in the spinal cord and trigeminal ganglia suggests that it could be targetable for the modulation of analgesia[52]. For example, $GABA_B$ receptors in dorsal reticular nucleus in the spinal cord are involved in inflammatory pain[14]. It has also been shown that $GABA_B$ receptor expression in neurons, astrocytes and microglia in the spinal cord dynamically changed in cancer-induced bone pain (CIBP) rat model, providing evidence that down-regulation of $GABA_B$ receptor contributes to

the development and maintenance of CIBP[53]. It has also been demonstrated that spinal $GABA_B$ receptor normalizes the N-methyl-D-aspartate receptor expression via the cAMP response element-binding protein, contributing to the diabetic neuropathic pain[54]. These studies further support the development of novel analgesics by targeting the $GABA_B$ receptor signaling.

In fact, it has been demonstrated as early as 1990s that systemic administration of (±) baclofen produced significant analgesic and antinociceptive effects in acute and chronic pain models, which can be blocked by the intrathecal $GABA_B$ receptor antagonists, but neither the $GABA_A$ receptor antagonist bicuculline nor the opioid receptor antagonist naloxone can block its effect, suggesting the involvement of $GABA_B$ receptor in this process[55]. In addition, intrathecal application of baclofen significantly attenuated CIBP-induced mechanical pain[53]. Consistent with the antinociceptive effect of $GABA_B$ receptor, the $GABA_{B1}$ knockout mice lacking GABA binding sites and $GABA_{B2}$ knockout mice lacking the functional $GABA_B$ receptors have been shown to exhibit hyperalgesia[56,57]. Thus, it is possible to develop $GABA_B$ receptor-specific analgesics, although their safety in long-term use is unclear.

3. Conclusion

In summary, GABA and its receptors are critically implicated in the transmission and regulation of pain. Among them, $GABA_A$ receptors play a role in analgesia mainly through two subtypes, $\alpha 2$ and $\alpha 3$, although the δ subtype may also be involved in pain modulation. In addition, $GABA_B$ receptors also seem to play a certain role in analgesia, which warrants further exploration. These evidence suggests the potential of targeting $GABA_A$ or $GABA_B$ receptor subtypes for the development of pain analgesics. Given the large number of receptor subtypes and interacting proteins that GABA interacts with, more fundamental research is needed to dissect the specific physiological and pharmacological roles of the targets of GABA. The emerging and promising evidence suggest that targeting GABAergic system could be a fruitful endeavor in the development of new efficacious analgesics in the clinical management of various painful conditions.

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References

1. Institute Of Medicine Us Committee On Advancing Pain Research, C.A.; Education. *Relieving pain in america: A blueprint for transforming prevention, care, education, and research*; National Academies Press (US): Washington (DC), **2011**.
2. Jensen, T.S.; Baron R; Haanpaa, M.; Kalso, E.; Loeser, J.D.; Rice, A.; Treede, R.D. A new definition of neuropathic pain. *Pain.* **2011**, *152*, 2204-2205.
3. Finnerup, N.B.; Kuner, R.; Jensen, T.S. Neuropathic pain: From mechanisms to treatment. *Physiol. Rev.* **2021**, *101*, 259-301.
4. van Hecke, O.; Austin, S.K.; Khan, R.A.; Smith, B.H.; Torrance, N. Neuropathic pain in the general population: A systematic review of epidemiological studies. *Pain.* **2014**, *155*, 654-662.
5. Finnerup, N.B.; Attal, N.; Haroutounian, S.; Mcnicol, E.; Baron R; Dworkin, R.H.; Gilron, I.; Haanpaa, M.; Hansson, P.; Jensen, T.S.; Kamerman, P.R.; Lund, K.; Moore, A.; Raja, S.N.; Rice, A.S.; Rowbotham, M.; Sena, E.; Siddall, P.; Smith, B.H.; Wallace, M. Pharmacotherapy for neuropathic pain in adults: A systematic review and meta-analysis. *Lancet Neurol.* **2015**, *14*, 162-173.
6. Kalso, E.; Aldington, D.J.; Moore, R.A. Drugs for neuropathic pain. *BMJ.* **2013**, *347*, f7339.
7. Baron R; Binder, A.; Wasner, G. Neuropathic pain: Diagnosis, pathophysiological mechanisms, and treatment. *Lancet Neurol.* **2010**, *9*, 807-819.
8. Bak, L.K.; Schousboe, A.; Waagepetersen, H.S. The glutamate/GABA-glutamine cycle: Aspects of transport, neurotransmitter homeostasis and ammonia transfer. *J. Neurochem.* **2006**, *98*, 641-653.
9. Barker, J.S.; Hines, R.M. Regulation of GABA(A) receptor subunit expression in substance use disorders. *Int. J. Mol. Sci.* **2020**, *21*.
10. Frolund, B.; Ebert, B.; Kristiansen, U.; Liljefors, T.; Krogsgaard-Larsen, P. GABA(A) receptor ligands and their therapeutic potentials. *Curr. Top. Med. Chem.* **2002**, *2*, 817-832.
11. Sarasa, S.B.; Mahendran, R.; Muthusamy, G.; Thankappan, B.; Selta, D.; Angayarkanni, J. A brief review on the non-protein amino acid, gamma-amino butyric acid (GABA): Its production and role in microbes. *Curr. Microbiol.* **2020**, *77*, 534-544.
12. Enna, S.J.; Mccarson, K.E. The role of GABA in the mediation and perception of pain. *Adv Pharmacol.* **2006**, *54*, 1-27.
13. Cellot, G.; Cherubini, E. GABAergic signaling as therapeutic target for autism spectrum disorders. *Front Pediatr.* **2014**, *2*, 70.
14. Martins, I.; Carvalho, P.; de Vries, M.G.; Teixeira-Pinto, A.; Wilson, S.P.; Westerink, B.; Tavares, I. GABA acting on GABAB receptors located in a medullary pain facilitatory area enhances nociceptive behaviors evoked by intraplantar formalin injection. *Pain.* **2015**, *156*, 1555-1565.
15. Braz, J.M.; Wang, X.; Guan, Z.; Rubenstein, J.L.; Basbaum, A.I. Transplant-mediated enhancement of spinal cord GABAergic inhibition reverses paclitaxel-induced mechanical and heat hypersensitivity. *Pain.* **2015**, *156*, 1084-1091.
16. Braz, J.M.; Sharif-Naeini, R.; Vogt, D.; Kriegstein, A.; Alvarez-Buylla, A.; Rubenstein, J.L.; Basbaum, A.I. Forebrain GABAergic neuron precursors integrate into adult spinal cord and reduce injury-induced neuropathic pain. *Neuron.* **2012**, *74*, 663-675.
17. Steiger, J.L.; Russek, S.J. GABAA receptors: Building the bridge between subunit mRNAs, their promoters, and cognate transcription factors. *Pharmacol Ther.* **2004**, *101*, 259-281.
18. Sieghart, W.; Fuchs, K.; Tretter, V.; Ebert, V.; Jechlinger, M.; Hoger, H.; Adamiker, D. Structure and subunit composition of GABA(A) receptors. *Neurochem. Int.* **1999**, *34*, 379-385.

19. Backus, K.H.; Arigoni, M.; Drescher, U.; Scheurer, L.; Malherbe, P.; Mohler, H.; Benson, J.A. Stoichiometry of a recombinant GABAA receptor deduced from mutation-induced rectification. *Neuroreport.* **1993**, *5*, 285-288.
20. Luo, Y.; Kusay, A.S.; Jiang, T.; Chebib, M.; Balle, T. Delta-containing GABA(A) receptors in pain management: Promising targets for novel analgesics. *Neuropharmacology.* **2021**, *195*, 108675.
21. Price, T.J.; Cervero, F.; de Koninck, Y. Role of cation-chloride-cotransporters (CCC) in pain and hyperalgesia. *Curr. Top. Med. Chem.* **2005**, *5*, 547-555.
22. Kahle, K.T.; Staley, K.J.; Nahed, B.V.; Gamba, G.; Hebert, S.C.; Lifton, R.P.; Mount, D.B. Roles of the cation-chloride cotransporters in neurological disease. *Nat Clin Pract Neurol.* **2008**, *4*, 490-503.
23. Willis, W.D. John Eccles' studies of spinal cord presynaptic inhibition. *Prog. Neurobiol.* **2006**, *78*, 189-214.
24. Garcia-Nicas, E.; Laird, J.M.; Cervero, F. GABAA-Receptor blockade reverses the injury-induced sensitization of nociceptor-specific (NS) neurons in the spinal dorsal horn of the rat. *J. Neurophysiol.* **2006**, *96*, 661-670.
25. Pirker, S.; Schwarzer, C.; Wieselthaler, A.; Sieghart, W.; Sperk, G. GABA(A) receptors: Immunocytochemical distribution of 13 subunits in the adult rat brain. *Neuroscience.* **2000**, *101*, 815-850.
26. Hortnagl, H.; Tasan, R.O.; Wieselthaler, A.; Kirchmair, E.; Sieghart, W.; Sperk, G. Patterns of mRNA and protein expression for 12 GABAA receptor subunits in the mouse brain. *Neuroscience.* **2013**, *236*, 345-372.
27. Uusi-Oukari, M.; Korpi, E.R. Regulation of GABA(A) receptor subunit expression by pharmacological agents. *Pharmacol. Rev.* **2010**, *62*, 97-135.
28. Wu, X.; Wu, Z.; Ning, G.; Guo, Y.; Ali, R.; Macdonald, R.L.; De Blas, A.L.; Luscher, B.; Chen, G. Gamma-Aminobutyric acid type a (GABAA) receptor alpha subunits play a direct role in synaptic versus extrasynaptic targeting. *J. Biol. Chem.* **2012**, *287*, 27417-27430.
29. Mckernan, R.M.; Rosahl, T.W.; Reynolds, D.S.; Sur, C.; Wafford, K.A.; Atack, J.R.; Farrar, S.; Myers, J.; Cook, G.; Ferris, P.; Garrett, L.; Bristow, L.; Marshall, G.; Macaulay, A.; Brown, N.; Howell, O.; Moore, K.W.; Carling, R.W.; Street, L.J.; Castro, J.L.; Ragan, C.I.; Dawson, G.R.; Whiting, P.J. Sedative but not anxiolytic properties of benzodiazepines are mediated by the GABA(A) receptor alpha1 subtype. *Nat. Neurosci.* **2000**, *3*, 587-592.
30. Crestani, F.; Low, K.; Keist, R.; Mandelli, M.; Mohler, H.; Rudolph, U. Molecular targets for the myorelaxant action of diazepam. *Mol. Pharmacol.* **2001**, *59*, 442-445.
31. Munro, G.; Ahring, P.K.; Mirza, N.R. Developing analgesics by enhancing spinal inhibition after injury: GABAA receptor subtypes as novel targets. *Trends Pharmacol. Sci.* **2009**, *30*, 453-459.
32. Knabl, J.; Witschi, R.; Hosl, K.; Reinold, H.; Zeilhofer, U.B.; Ahmadi, S.; Brockhaus, J.; Sergejeva, M.; Hess, A.; Brune, K.; Fritschy, J.M.; Rudolph, U.; Mohler, H.; Zeilhofer, H.U. Reversal of pathological pain through specific spinal GABAA receptor subtypes. *Nature.* **2008**, *451*, 330-334.
33. Mirza, N.R.; Larsen, J.S.; Mathiasen, C.; Jacobsen, T.A.; Munro, G.; Erichsen, H.K.; Nielsen, A.N.; Troelsen, K.B.; Nielsen, E.O.; Ahring, P.K. NS11394 [3'-[5-(1-hydroxy-1-methyl-ethyl)-benzoimidazol-1-yl]-biphenyl-2-carbonitrile], a unique subtype-selective GABAA receptor positive allosteric modulator: In vitro actions, pharmacokinetic properties and in vivo anxiolytic efficacy. *J. Pharmacol. Exp. Ther.* **2008**, *327*, 954-968.
34. Munro, G.; Lopez-Garcia, J.A.; Rivera-Arconada, I.; Erichsen, H.K.; Nielsen, E.O.; Larsen, J.S.; Ahring, P.K.; Mirza, N.R. Comparison of the novel subtype-selective GABAA receptor-positive allosteric modulator NS11394 [3'-[5-(1-hydroxy-1-methyl-ethyl)-benzoimidazol-1-yl]-biphenyl-2-carbonitrile] with diazepam,

zolpidem, bretazenil, and gaboxadol in rat models of inflammatory and neuropathic pain. *J. Pharmacol. Exp. Ther.* **2008**, *327*, 969-981.

35. Scott-Stevens, P.; Atack, J.R.; Sohal, B.; Worboys, P. Rodent pharmacokinetics and receptor occupancy of the GABAA receptor subtype selective benzodiazepine site ligand L-838417. *Biopharm. Drug Dispos.* **2005**, *26*, 13-20.
36. Atack, J.R.; Wafford, K.A.; Tye, S.J.; Cook, S.M.; Sohal, B.; Pike, A.; Sur, C.; Melillo, D.; Bristow, L.; Bromidge, F.; Ragan, I.; Kerby, J.; Street, L.; Carling, R.; Castro, J.L.; Whiting, P.; Dawson, G.R.; Mckernan, R.M. TPA023 [7-(1,1-dimethylethyl)-6-(2-ethyl-2H-1,2,4-triazol-3-ylmethoxy)-3-(2-fluorophenyl)-1,2,4-triazolo[4,3-b]pyridazine], an agonist selective for alpha2- and alpha3-containing GABAA receptors, is a nonsedating anxiolytic in rodents and primates. *J. Pharmacol. Exp. Ther.* **2006**, *316*, 410-422.
37. Nickolls, S.; Mace, H.; Fish, R.; Edye, M.; Gurrell, R.; Ivarsson, M.; Pitcher, T.; Tanimoto-Mori, S.; Richardson, D.; Sweatman, C.; Nicholson, J.; Ward, C.; Jinks, J.; Bell, C.; Young, K.; Rees, H.; Moss, A.; Kinloch, R.; Mcmurray, G. A comparison of the alpha2/3/5 selective positive allosteric modulators l-838,417 and TPA023 in preclinical models of inflammatory and neuropathic pain. *Adv Pharmacol Sci.* **2011**, *2011*, 608912.
38. Di Lio, A.; Benke, D.; Besson, M.; Desmeules, J.; Daali, Y.; Wang, Z.J.; Edwankar, R.; Cook, J.M.; Zeilhofer, H.U. HZ166, a novel GABAA receptor subtype-selective benzodiazepine site ligand, is antihyperalgesic in mouse models of inflammatory and neuropathic pain. *Neuropharmacology.* **2011**, *60*, 626-632.
39. Ralvenius, W.T.; Benke, D.; Acuna, M.A.; Rudolph, U.; Zeilhofer, H.U. Analgesia and unwanted benzodiazepine effects in point-mutated mice expressing only one benzodiazepine-sensitive GABAA receptor subtype. *Nat. Commun.* **2015**, *6*, 6803.
40. Farrant, M.; Nusser, Z. Variations on an inhibitory theme: Phasic and tonic activation of GABA(A) receptors. *Nat. Rev. Neurosci.* **2005**, *6*, 215-229.
41. Peng, H.Y.; Chen, G.D.; Lee, S.D.; Lai, C.Y.; Chiu, C.H.; Cheng, C.L.; Chang, Y.S.; Hsieh, M.C.; Tung, K.C.; Lin, T.B. Neuroactive steroids inhibit spinal reflex potentiation by selectively enhancing specific spinal GABA(A) receptor subtypes. *Pain.* **2009**, *143*, 12-20.
42. Iura, A.; Takahashi, A.; Hakata, S.; Mashimo, T.; Fujino, Y. Reductions in tonic GABAergic current in substantia gelatinosa neurons and GABA(A) receptor delta subunit expression after chronic constriction injury of the sciatic nerve in mice. *Eur. J. Pain.* **2016**, *20*, 1678-1688.
43. Ataka, T.; Gu, J.G. Relationship between tonic inhibitory currents and phasic inhibitory activity in the spinal cord lamina II region of adult mice. *Mol. Pain.* **2006**, *2*, 36.
44. Brown, N.; Kerby, J.; Bonnert, T.P.; Whiting, P.J.; Wafford, K.A. Pharmacological characterization of a novel cell line expressing human alpha(4)beta(3)delta GABA(A) receptors. *Br J Pharmacol.* **2002**, *136*, 965-974.
45. Bonin, R.P.; Labrakakis, C.; Eng, D.G.; Whissell, P.D.; De Koninck, Y.; Orser, B.A. Pharmacological enhancement of delta-subunit-containing GABA(A) receptors that generate a tonic inhibitory conductance in spinal neurons attenuates acute nociception in mice. *Pain.* **2011**, *152*, 1317-1326.
46. Szelenyi, I. Flupirtine, a re-discovered drug, revisited. *Inflamm. Res.* **2013**, *62*, 251-258.
47. Kornhuber, J.; Bleich, S.; Wiltfang, J.; Maler, M.; Parsons, C.G. Flupirtine shows functional NMDA receptor antagonism by enhancing Mg2+ block via activation of voltage independent potassium channels. Rapid communication. *J Neural Transm (Vienna).* **1999**, *106*, 857-867.

 doi:10.20944/preprints202305.1444.v1

48. Klinger, F.; Bajric, M.; Salzer, I.; Dorostkar, M.M.; Khan, D.; Pollak, D.D.; Kubista, H.; Boehm, S.; Koenig, X. Delta Subunit-containing GABAA receptors are preferred targets for the centrally acting analgesic flupirtine. *Br J Pharmacol.* **2015**, *172*, 4946-4958.
49. Benke, D. GABA(B) receptors and pain. *Curr Top Behav Neurosci.* **2022**, *52*, 213-239.
50. Terunuma, M. Diversity of structure and function of GABA(B) receptors: A complexity of GABA(B)-mediated signaling. *Proc Jpn Acad Ser B Phys Biol Sci.* **2018**, *94*, 390-411.
51. Malcangio, M. GABA(B) receptors and pain. *Neuropharmacology.* **2018**, *136*, 102-105.
52. Antonopoulos, S.R.; Durham, P.L. Grape seed extract suppresses calcitonin gene-related peptide secretion and upregulates expression of GAD 65/67 and GABAB receptor in primary trigeminal ganglion cultures. *IBRO Neurosci Rep.* **2022**, *13*, 187-197.
53. Zhou, Y.Q.; Chen, S.P.; Liu, D.Q.; Manyande, A.; Zhang, W.; Yang, S.B.; Xiong, B.R.; Fu, Q.C.; Song, Z.P.; Rittner, H.; Ye, D.W.; Tian, Y.K. The role of spinal GABAB receptors in Cancer-Induced bone pain in rats. *J. Pain.* **2017**, *18*, 933-946.
54. Bai, H.P.; Liu, P.; Wu, Y.M.; Guo, W.Y.; Guo, Y.X.; Wang, X.L. Activation of spinal GABAB receptors normalizes N-methyl-D-aspartate receptor in diabetic neuropathy. *J. Neurol. Sci.* **2014**, *341*, 68-72.
55. Malcangio, M.; Ghelardini, C.; Giotti, A.; Malmberg-Aiello, P.; Bartolini, A. CGP 35348, a new GABAB antagonist, prevents antinociception and muscle-relaxant effect induced by baclofen. *Br J Pharmacol.* **1991**, *103*, 1303-1308.
56. Schuler, V.; Luscher, C.; Blanchet, C.; Klix, N.; Sansig, G.; Klebs, K.; Schmutz, M.; Heid, J.; Gentry, C.; Urban, L.; Fox, A.; Spooren, W.; Jaton, A.L.; Vigouret, J.; Pozza, M.; Kelly, P.H.; Mosbacher, J.; Froestl, W.; Kaslin, E.; Korn, R.; Bischoff, S.; Kaupmann, K.; van der Putten, H.; Bettler, B. Epilepsy, hyperalgesia, impaired memory, and loss of pre- and postsynaptic GABA(B) responses in mice lacking GABA(B(1)). *Neuron.* **2001**, *31*, 47-58.
57. Gassmann, M.; Shaban, H.; Vigot, R.; Sansig, G.; Haller, C.; Barbieri, S.; Humeau, Y.; Schuler, V.; Muller, M.; Kinzel, B.; Klebs, K.; Schmutz, M.; Froestl, W.; Heid, J.; Kelly, P.H.; Gentry, C.; Jaton, A.L.; Van der Putten, H.; Mombereau, C.; Lecourtier, L.; Mosbacher, J.; Cryan, J.F.; Fritschy, J.M.; Luthi, A.; Kaupmann, K.; Bettler, B. Redistribution of GABAB(1) protein and atypical GABAB responses in GABAB(2)-deficient mice. *J. Neurosci.* **2004**, *24*, 6086-6097.