

Targeting GABA to Cure Anxiety Disorder in Various Methods

Yihui Lu^{1,†}^a, Jiayi Yuan^{2,†}^b and Xichen Zhang^{3,†}^c

¹Environmental and Life Sciences Faculty University of Southampton, Southampton, U.K.

²Qingdao Chengyang No.1 Senior High School, Qingdao, China

³Shanghai Shangde Experimental School, Shanghai, China

[†]These authors contributed equally

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Abstract: The number of people suffering from anxiety disorders has risen sharply in recent years. Gamma-aminobutyric acid (GABA) is a neurotransmitter in the central nervous system that inhibits (prevents) nerve activity by restricting nerve transmission. A large number of studies have shown that low GABA levels or GABA system dysfunction can cause anxiety. This article mainly summarizes the treatment and mechanism of anxiety disorders for GABA metabolic uptake, GABAA receptor, and GABAB receptor. The metabolic process of GABA was outlined, by using drugs that increase the expression of GAD, inhibit GABA-T, and block GAT to increase the content of GABA and treat anxiety. The structure of GABAA receptors and how benzodiazepine targets GABAA receptors to treat anxiety disorders were detailed. Finally, anxiety disorders can be treated by GABAB receptor agonist baclofen and positive allosteric modulators (PAMs).

1 INTRODUCTION

Anxiety is a common unpleasant emotional state marked by emotions of fear and dread, as well as distinct physical, cognitive, and behavioral symptoms. Anxiety is a normal element of one's behavioral repertoire, and it can be useful as a protective mechanism for increasing awareness and response to unexpected situations. However, when it is overly intense or frequent, or when it occurs in inappropriate situations, it can interfere with regular functioning and thus be called abnormal (Roy-Byrne, 2005). The people who are in this state are suffering from an anxiety disorder. Anxiety disorder is a major mental health problem that affects people all over the world and the proportion of people who are suffering from this disease are increasing. In a 2020 survey, 62 percent of respondents said they were anxious in some way (Team 2021). And the proportion of those who are suffering from an anxiety disorder is increasing year after year. The global prevalence of all mental disorders increased by 50% between 1990 and 2013, from 416 million to 615 million persons

(World Health Organizations 2021). Besides, Anxiety disorders impacted around 273 million people worldwide in 2010. In 2017, an estimated 264 million people worldwide suffered from anxiety (Figure 1). Thus, there are such a large number of people are struggling with this disease, our research aims to provide a great number of meaningful methods to relieve symptoms to some extent.

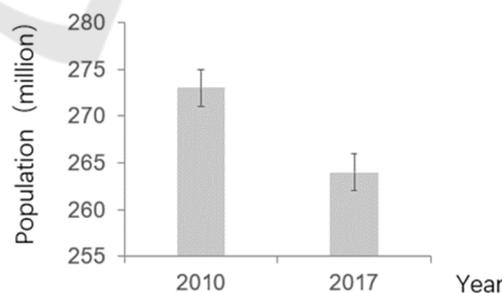


Figure 1: The number of mental disorder patients (World Health Organizations, 2021).

Gamma-aminobutyric acid (GABA), which is found in more than a third of central nervous system

^a <https://orcid.org/0000-0003-3963-7140>

^b <https://orcid.org/0000-0001-8315-7609>

^c <https://orcid.org/0000-0003-2712-1091>

(CNS) synapses, is one of the most widely distributed neurotransmitters in the brain (Peter P Roy-Byrne 2021). The etiology of anxiety disorders is related to changes in the GABA system. Allosteric sites on the GABA receptor allow for precise modulation of the level of inhibition of neurons in the amygdala, and these sites are the molecular targets of the most common types of anxiolytic medicines (Gauthier 2015).

To explain the content of GABA and the treatment of anxiety, we first detailed the metabolic process of GABA by employing medications that boost the expression of GAD, inhibit GABA-T, and block GAT. The structure of GABA receptors was then discussed, as well as how benzodiazepines target GABA receptors to treat anxiety disorders. Finally, anxiety disorders can be treated using the GABA receptor agonist baclofen and positive reinforcement.

2 GABA METABOLISM

GABA transport, sequestration, synthesis, and degradation are all mediated by various specific molecular pathways (Roth 2021). GABA metabolism and uptake both play a role in the factors that influence anxiety pathogenesis (Kalueff, 2007).

The enzyme glutamic acid decarboxylase (GAD) and the cofactor pyridoxal phosphate are used to convert glutamate, the principal excitatory neurotransmitter, into GABA in the GABAergic neurons of the central nervous system. Only cells that employ GABA as a neurotransmitter produce GAD. GABA is packaged into vesicles by vesicle GABA transporters (VGAT), released into the synaptic cleft, and diffused across the cleft to the target receptors located on the postsynaptic surface when the presynaptic neuron is depolarized. Furthermore, Presynaptic terminals and surrounding glial cells can resorb GABA released into the synapse cleft for a variety of objectives. Membrane GABA transporters (GAT) allow GABA to be re-used in presynaptic terminals, however GABA in glial cells is converted to succinic semialdehyde by GABA-T and cannot be re-synthesised in this compartment because glia lack GAD. Through a circuitous route that involves the Krebs cycle, GABA can eventually be recovered from this source. GABA is transformed to glutamine in glia by the GABA shunt, which subsequently transports glutamine back to presynaptic neurons, where glutamine is converted to glutamate by glutaminase. Anxiety is dependent on GABA metabolism, which may be disrupted in the pathophysiology (Siegel 1999).

2.1 Glutamic Acid Decarboxylase

The enzyme glutamic acid decarboxylase (GAD) is responsible for converting glutamate to GABA. GAD comes in two molecular forms in the mammalian brain: Glutamic acid decarboxylase 67 (GAD67) and Glutamic acid decarboxylase (GAD65), which are produced by two independently regulated genes, GAD1 and GAD2, respectively (Tillakaratne, 1995) (Hetteema 2006). In terms of intraneuronal expression, GAD65 appears to be more restricted to axon terminals, whereas GAD67 appears to be more equally distributed throughout the neuron. Furthermore, the active cofactor-bound holoenzyme form of GAD67 makes up nearly all of the enzyme, whereas majority of GAD65 is found as a reserve pool of inactive apoenzyme. One study suggests that acute stress may enhance GAD67 production, whereas chronic stress may increase GAD65 availability (Hetteema 2006).

Mice missing a short 65-kDa GAD isoform (GAD65), which is responsible for fine-tuning GABAergic neurotransmission, GABA levels were lower and anxiety levels were higher, whereas AD reboxetine increased GAD65 expression in the septum of stressed rats. Recent research has discovered a relationship has been discovered between a GAD65 gene polymorphism and anxiety-related behavioral inhibition in children, as well as lower levels of GAD65 and GAD67 in the prefrontal cortex and cerebellum in depressed people (Kalueff 2007). In human studies, neurotic patients and those with mood disorders showed lower GAD plasma activity. In a small family-based study of children, researchers discovered a small link between GAD2 and behavioral inhibition, and anxiety-related traits (Hetteema 2006). Therefore, a decrease in GAD level will result in less synthesis of GABA, which will lead to increased anxiety.

2.2 GABA Transaminase

Another important enzyme in GABA turnover is GABA transaminase (GABA-T), which catabolizes GABA (Kalueff 2007). GABA-T inhibition increases brain levels of the main inhibitory neurotransmitter GABA, which has been linked to a range of functional consequences, including behavioral alterations (Sherif 1995). Its inhibitors, such as Vigabatrin (gamma-vinyl GABA, GVG), can raise brain GABA levels by irreversibly blocking GABA-T. In the plus-maze test, treatment with vigabatrin at a dose of 250 mg/kg, i.p. lowered anxiety levels in both groups of differentially housed rats

(Tillakaratne, 1995). Inhibiting GABA-T, elevating brain GABA levels, producing anxiolytic-like effects in animals, and modulating glutamate and catecholamine neurotransmission are all effects of the AD phenelzine (In humans, it is beneficial in the treatment of social anxiety and panic disorders) (Kalueff 2007). In various experimental models, phenylethylidenehydrazine (PEH), an derivative of the monoamine oxidase inhibitor beta-phenylmethyl hydrazine (phenelzine), which inhibits the gamma-aminobutyric acid (GABA) catabolic enzyme GABA-transaminase and elevates GABA levels in the brain and has strong anxiolytic properties (Duffy 2004).

2.3 GABA Transporter

GABA transporters (GATs) are important molecules in the transfer of GABA and are found on the membrane of cells. At the synapse, GATs can regulate the duration and intensity of GABAergic activity by reabsorbing GABA. GAT1, GAT2, GAT3, and GAT4 are among the GABA transporter subtypes discovered. GAT1 is the most common subtype in the brain, and it can be found in both synaptic and extrasynaptic sites (Liu 2007). It's also in charge of maintaining 75 percent of synaptic GABA concentration and delivering it to GABAA receptors to initiate receptor-mediated postsynaptic neuron inhibition. GAT1 is largely involved in GABA binding and transport from the cytoplasm to the extracellular space (reverse mode) and back (forward mode). As a result, if GAT1 is malfunctioning, communication with postsynaptic GABA receptors may be delayed (Zafar 2018). GAT1 deficiency results in increased extracellular GABA levels and GABAA receptor overactivation. Tremor, ataxia, and nervousness are all symptoms of GAT1 deficiency, according to behavioral tests. GAT1 is involved in the development of anxiety disorders, according to several behavioral tasks such as the tail-suspension test, forced swim test, and open-field test. Tiagabine hydrochloride is a selective GABA reuptake inhibitor that blocks GAT1 and hence raises GABA tone. It is used to treat anxiety disorders. For decades, GAT1 has been recognized as a potential therapeutic target due to its critical role in the GABAergic transport mechanism (Liu 2007).

2.4 Neuropeptide

Neuropeptides are small proteins that operate as neuronal signaling molecules and have a role in a variety of brain functions, including analgesia,

reward systems, social behaviors, learning, and memory (Garakani 2020). It's also important to consider neuropeptides' role in modulating GABAergic function and anxiety/depression interplay. Melatonin, for example, has been shown in animals to have both anxiolytic and anti-properties (Kalueff 2007). Melatonin improved GABAergic inhibitory transmission by increasing the amplitude and frequency of GABAergic mIPSCs. As a result, we've discovered that melatonin boosts the GABAergic system's performance (Cheng 2021). Cholecystokinin antagonists are neuropeptides that regulate the GABAergic system and is implicated in both anxiety pathogenesis and treatment, have been found to have similar properties (Kalueff 2007). Oxytocin has been associated to high anxiety in healthy individuals and has been proven to have a good influence on emotion modulation in healthy adults. Animal research imply that oxytocin has anxiolytic qualities, however human studies suggest that oxytocin can produce acute anxiety. In animal models, arginine vasopressin (AVP) has been linked to anxiety, anxiolytic properties may be found in vasopressin V1a and V1b receptor antagonists. Despite the fact that neuropeptides appear to be a promising new therapy option for anxiety disorders, no specific treatment candidates have been identified (Garakani 2020).

3 GABAA RECEPTOR

The GABAA receptor is one of the most common types of receptor for the inhibitory neurotransmitter gamma-aminobutyric acid (GABA) (OLSEN, Tobin 1990). GABAA receptors are made up of five protein subunits that bridge a lipid bilayer to produce a cylindrical structure. There is a ligand-gated ion channel in the center. (Roy-Byrne 2005) (OLSEN, Tobin 1990) (Bruce 2021). It allows chloride ions to move in and move out, using this mechanism, it can regulate excitability. And there are many agonists and antagonists to accommodate the process of GABA binding with GABAA receptor, which plays role in curing anxiety disorders, like benzodiazepines.

3.1 The Structure of GABAA Receptor

Electric organs of electric rays and eels, as well as vertebrate skeletal muscle, contain GABAA receptors. All are pentameric oligomers with a mass of around 250 kilodaltons (kDa) and four different types of subunits, each with a mass of about 50 kDa. GABAA receptors in the central nervous system of

vertebrates appear to be made up of solely α , β and γ polypeptides, with a total of four or five subunits. The oligomeric subgroups differ in terms of developmental stage, tissue type, and brain region, as well as pharmacological qualities (OLSEN, Tobin 1990). As a result, the GABAA receptor is a pentamer. It's also a heteropentamer, according to a number of studies. There are several molecular families of subunits that have been found, including those α with 6 isoforms, β with 3 isoforms, γ with 3 isoforms, θ with 1 isoform, and ρ with 3 isoforms (Tallman 2002). Furthermore, when viewed from the extracellular space, the receptor complex is known to consist of 2 α subunits alternating with 2 β subunits ordered b-a-b-g-a in a clockwise orientation and a single γ subunit (Sherif 1995) (Duffy 2004) (Liu 2007) (Zafar 2018). The tri-heteromeric receptor, which consists of two α , two β , and one γ subunit, in the vertebrate brain, is the most common subunit combination, despite the fact that there are multiple possible layouts (Chang 1996) (Farrar 1999) (Tretter 1997).

3.2 Ligand-gated Channel

There are two GABA binding sites in each receptor complex, but only one benzodiazepine binding site. The GABA binding sites are found at the junction of the two β subunit pairs that alternate, while the lone benzodiazepine binding site is found at the crossroads of the single α and γ subunit pairings (Roy-Byrne 2005). There are many different subtypes to choose from due to the numerous diverse subunits and their arrangements, each with its own affinity for GABA, chloride channel kinetics, and affinity for different benzodiazepine ligands (Roy-Byrne 2005). The vast majority of these receptor complexes, according to evidence, are made up of $\alpha 1$ subunit in combination with $\beta 2$ and $\gamma 2$ subunits (Mohler HF 2001). The $\alpha 2$ or $\alpha 3$ receptor subtype is likely to be responsible for benzodiazepine sedation (Rudolph U 1999) (Lydiard 2003).

3.3 GABAA Receptor Channel

There are GABAA receptors play a critical role in balancing excitatory transmission. When activated by GABA, the protein subunits undergo conformational changes, resulting in the brief development of a channel along the cylinder's axis via which chloride ions can flow from the outside to the interior (Kalueff 2007). The chloride ions flowing into the cytoplasm will cause hyperpolarization, so GABA is considered an inhibitory neurotransmitter. As a result, GABA is

known to counteract the excitatory neurotransmitter glutamate's effect (Tillakaratne 1995).

3.4 Pharmacology

The GABA-benzodiazepine receptor, also known as the GABAA receptor or the benzodiazepine receptor, may play a role in the pathophysiology of anxiety as well as its treatment (Figure 2). GABAA receptors are important since they are where benzodiazepines act. It refers to a complex glycoprotein that has binding sites for a variety of benzodiazepine drugs with potent anxiolytic properties (Roy-Byrne 2005). The reason why benzodiazepine can be used to treat anxiety disorder on GABAA receptor is that benzodiazepines allosterically modulated GABAA receptors, which are used for their sedative, anxiolytic, anticonvulsant, and muscle relaxant actions (Rudolph 2018). To be specific, the neurotransmitter GABA opens the chloride channel when it binds to GABAA receptor while benzodiazepines control this opening (Sigel 2018). Furthermore, benzodiazepines influence this channel opening triggered by either agonist binding site (Baur 2005).

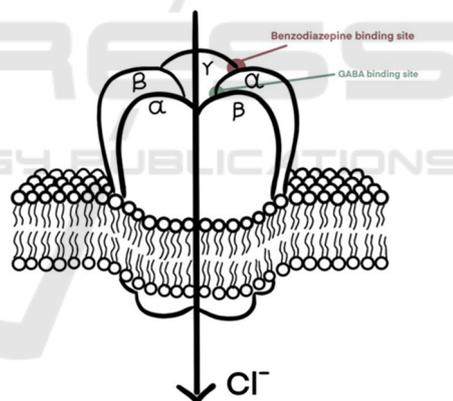


Figure 2: The Structure of GABAA receptor.

Benzodiazepines are effective as a drug to treat anxiety disorder when they bind with GABAA receptors containing specific subunits. The most sophisticated drugs, which target GABAA receptors with $\alpha 2$ and $\alpha 3$ (positive allosteric modulation) and $\alpha 5$ subunits (negative allosteric modulation), are now being evaluated in clinical trials for anxiolytic effects, and using one subunit (negative allosteric modulation) to avoid functional effects at GABAA receptors, while using only $\alpha 1$ subunit to avoid functional effects at GABAA receptors (Rudolph 2018). In addition to their anxiolytic effect, which is

mediated by $\alpha 2$ and potentially also by $\alpha 3$ -containing GABAA receptors, benzodiazepines exhibit sedative properties mediated by $\alpha 1$ -containing GABAA receptors (Rudolph, 2018). Benzodiazepines are only sensitive to receptor assemblies that have an $\alpha 1$ subunit next to $\gamma 2$ (Minier 2004). As a result, when, benzodiazepines combine with different sub-type of GABAA receptors, they have sedative, hypnotic, muscle relaxant, and anticonvulsant properties, with extremely minimal risk of overdosing (Sigel 2018).

4 GABAB RECEPTOR

The GABAB receptor, which is a G-protein-coupled receptor, suppresses adenylate cyclase activity and mediates synaptic inhibition's gradual and sustained component (Bowerly N 2004). GABAB receptors, highly expressing in the limbic system are, were found in almost all neuronal cells, especially GABAB (1) receptor. GABAB (1) and GABAB (2) are two subunits of the GABAB receptor that heterodimerize to create the functional GABAB receptor. The orthosteric ligand binding site is found in the GABAB (1) subunit, whereas the GABAB (2) subunit, responsible for G-protein activation, contains positive allosteric modulator binding sites (Gassmann M 2012).

4.1 Baclofen

Baclofen was a powerful and selective agonist of GABAB receptor in 1980, and it was shown that baclofen inhibited neurotransmitter release in the central nervous system when it acted on it. GABAB receptor agonists, including baclofen, have a lot of preclinical evidence that they could be useful in the treatment of anxiety disorders (Felice D 2016) (Vinkers CH 2010). Baclofen, the first known GABA derivative, was created in 1962 by combining a halogenated phenylring with carbon to create a molecule that could cross the blood-brain barrier (BBB). The functions of baclofen are mainly used as a muscle relaxant and antispastic. Research of baclofen revealed a number of drawbacks: it couldn't passively penetrate the BBB and had a short duration of action and rapid tolerance development in the patient (Felice 2020).

Efforts have been made to obtain baclofen analogs, but no superior drug options have emerged. One of these compounds was phenibut, which was made by merely removing the chlorine atom from baclofen. The molecule has anxiolytic and nootropic properties, however it was quickly determined that it

was not GABAB receptor-selective. Other experiments attempted but failed, to rigidify the baclofen structure by inserting groups like ethylene and propylene. Some efforts to obtain baclofen analogs resulted in clinically authorized medications, such as pregabalin, vigabatrin, and gabapentin, although these were discovered to act through distinct processes and bind receptors other than the GABAB receptor. Although some inconsistent outcomes have been reported, baclofen treatment has been demonstrated to attenuate anxiety-like behavior in numerous rat models and mouse models. For example, one study found that baclofen was useful in the Vogel conflict test, whereas another found that it had no impact (Lu Y 2016). The side effects of Baclofen included muscular relaxation, sedation, vertigo, somnolence, and hypothermia (Agabio 2013). PAMs, one of GABAB receptors, have a lower risk of receptor desensitization/tolerance as compared to baclofen, which was typical GABAB receptor agonists.

Table 1: The typical medicine, target receptor, mechanism and side-effects.

Name of Medicine	Target receptor/Mechanism	Side-effect(s)
Reboxetine	Increased GAD65 expression	Dry mouth, Constipation.
Vigabatrin	GABA-T inhibitor, raise brain GABA levels by irreversibly blocking GABA-T	Problems walking or feeling uncoordinated, feeling dizzy, shaking, joint pain.
Phenylethylidenehydrazine (PEH)	Inhibit GABA-T	No clinical trials, thus no known side-effect.
Tiagabine hydrochloride	Selective GABA-reuptake inhibitor, increases GABA tone via GAT1 blockade	Inability to concentrate, dizziness, drowsiness, nervousness, irritability.
Antagonists of cholecystokinin	Blocks the receptor sites for the peptide hormone cholecystokinin (CCK), a neuropeptide that modulates the GABAergic system	Nausea, vomiting, constipation, drowsiness.
Benzodiazepine	GABAA	Dependence, rebound anxiety, memory impairment, and discontinuation syndrome.
Baclofen	GABAB	Short duration of action and rapid tolerance development.
Positive Allosteric modulators (PAMs)	GABAB	Addiction liability and respiratory depression.

4.2 Positive Allosteric Modulators (PAMs)

Compared with receptor agonists such as baclofen, PAMs have several advantages: (1) PAM binding causes receptor potentiation rather than direct activation; (2) When the allosteric binding site is saturated, the target receptor will not be down-regulated or over-stimulated (Gjoni T 2008) (Gjoni 2009). GABAB receptor agonists, such as PAMs, will produce anxiolytic-like effects, whereas defunction of GABAB receptor (in GABAB (1) and GABAB (2) animals models) will produce anxiogenic-like effects. Losing the function of either the GABAB (1a, 2) or GABAB (1b, 2) receptor subunit isoform alone, however, exhibited no effect on anxiety-like behavior in these mice, most likely because they still retained functioning GABAB receptors (GABAB(1b,2) or GABAB(1a,2), respectively). GABAB receptor antagonists have an unknown effect on anxiety, however they appear to be anxiolytic in some situations, similar to agonists/PAMs. The exact mechanism of the anxiolytic effects of GABAB receptor antagonists and agonists/PAMS, which should have opposing pharmacological actions, are still unknown. This could be because GABAB receptors are located both pre- and post-synaptically, and medicines' efficacy and timings may differ (Freyd T 2017). Despite this, data suggested that the GABAB receptor could be a promising therapeutic target for anxiety disorders. Moreover, the typical treatments for anxiety disorders are as long as their respective characteristics, as shown in Table 1.

5 CONCLUSIONS

There are various methods of anxiety treatment by using GABA. Includes modulate GABA metabolism, regulating GABAA and GABAB receptor. There are several ways of GABA regulations such as GAD, which could increase the expression of GABA-related gene and increase the amount of GABA, then the number of receptor increases which cause anxiety to be inhibited. The second way is to use a GABA transporter, which could inhibit the metabolism of GABA, then the number of GABA increases, and result in the rise of GABA level. GAT also plays an important role, which regulates the duration and intensity of GABAergic activity presynaptically and postsynaptically. These are the method that introduced in modulating GABA metabolism. GABAA is crucial, and the main therapeutic target in

anxiety disorder. Benzodiazepine is an essential part of anxiety. It reacts and combines with specific subunits in the GABAA receptor which ultimately reduces anxiety. GABAB is also a significant but less developed receptor to cure anxiety. Baclofen which is a GABAB agonist and PAM increases the effect of enzymes which could ultimately decrease the level of anxiety. However, recent studies and research shows there is a sea of side effects and obstacles during therapy. As benzodiazepine can cause sleep and baclofen had a short duration of action and rapid tolerance development. Fixing these problems is the real question that is facing and hope to fix in the future.

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