

REVIEW

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GABA_A receptors: structure, function, pharmacology, and related disorders



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Abstract

Background: γ -Aminobutyric acid sub-type A receptors (GABA_ARs) are the most prominent inhibitory neurotransmitter receptors in the CNS. They are a family of ligand-gated ion channel with significant physiological and therapeutic implications.

Main body: GABA_ARs are heteropentamers formed from a selection of 19 subunits: six α (alpha1-6), three β (beta1-3), three γ (gamma1-3), three ρ (rho1-3), and one each of the δ (delta), ϵ (epsilon), π (pi), and θ (theta) which result in the production of a considerable number of receptor isoforms. Each isoform exhibits distinct pharmacological and physiological properties. However, the majority of GABA_ARs are composed of two α subunits, two β subunits, and one γ subunit arranged as $\gamma 2\beta 2\alpha 1\beta 2\alpha 1$ counterclockwise around the center. The mature receptor has a central chloride ion channel gated by GABA neurotransmitter and modulated by a variety of different drugs. Changes in GABA synthesis or release may have a significant effect on normal brain function. Furthermore, The molecular interactions and pharmacological effects caused by drugs are extremely complex. This is due to the structural heterogeneity of the receptors, and the existence of multiple allosteric binding sites as well as a wide range of ligands that can bind to them. Notably, dysfunction of the GABAergic system contributes to the development of several diseases. Therefore, understanding the relationship between GABA_A receptor deficits and CNS disorders thus has a significant impact on the discovery of disease pathogenesis and drug development.

Conclusion: To date, few reviews have discussed GABA_A receptors in detail. Accordingly, this review aims to summarize the current understanding of the structural, physiological, and pharmacological properties of GABA_ARs, as well as shedding light on the most common associated disorders.

Keywords: GABA, GABA_AR, Benzodiazepine, Barbiturates, Allosteric modulation, Autism spectrum disorder, Alzheimer's disease, Epilepsy, Schizophrenia

Background

γ -Aminobutyric acid (GABA), the primary inhibitory neurotransmitter in the central nervous system (CNS), is a key coordinator of brain activity. GABA's inhibitory effects are mediated by two types of receptors, GABA_A and GABA_B receptors [1]. GABAergic neurotransmission is critical in neurodevelopmental disorders [2]. GABA_AR is one of the most significant drug targets in

the treatment of neuropsychiatric disorders such as epilepsy, insomnia, and anxiety, as well as in anesthesia in surgical operations [3]. In addition, genetic studies have documented the relationship between GABA_AR subunit genes and epilepsy [4], eating disorder [5], autism [6, 7], and bipolar disorders [8]. GABA_B receptors are members of the C family of G protein-coupled receptors (GPCRs), which are found in the nervous system and have been linked to some neurological and psychiatric disorders [9]. They are structurally and functionally distinct from GABA_A receptors and will not be covered in this article. GABA_C receptors are now considered to be part of GABA_A receptor isoforms that are entirely made up of

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rho (ρ) subunits [10]. In this review, we will try to provide a quick rundown of what we know about GABA_A receptors, including their structure, function, pharmacology, and related disorders.

GABA_ARs structure and gene organization

GABA_A receptors are ligand-gated chloride channels that consist of pentameric combinations of different subunits. A total of 19 GABA_A receptor subunit genes have been identified in humans that code for six α (alpha1-6), three β (beta1-3), three γ (gamma1-3), three ρ (rho1-3), and one each of the δ (delta), ϵ (epsilon), π (pi), and θ (theta) (Fig. 1A; Table 1) [11–13]. The diversity of GABA_A receptors is due to the alternative splicing of several genes [14]. The GABA_A receptor subunit genes are mainly arranged into four clusters on the human genome's chromosomes 4, 5, 15, and X. Four genes, α 2, α 4, β 1, and γ 1 on chromosome 4; four genes α 1, α 6, β 2, and γ 2 on chromosome 5; three genes, α 5, β 3, and γ 3 on chromosome 15; and three genes, α 3, ϵ , and θ on

chromosome X (Table 1) [15]. The receptor composition and arrangement influence its functional and pharmacological properties [16, 17].

Each subunit has been thoroughly investigated in terms of amino acid sequence, level of expression, and localization in brain tissues, but it is still unclear the interaction between them to form many different isoforms [18]. This variety of isoforms may be present even in a single cell [10]. However, it is widely assumed that the main adult isoform is composed of α 1, β 2, and γ 2 subunits which are arranged γ 2 β 2 α 1 β 2 α 1 counterclockwise around a central pore as viewed from the cell exterior (Fig. 1B) [19].

GABA_AR subunits share a common structure (Fig. 1A). The mature subunit is composed of ~450 amino acid residues. It contains N-terminal, a large hydrophilic extracellular domain (ECD), four hydrophobic transmembrane domains (TMD: TM1–TM4) where TM2 is believed to form the pore of the chloride channel, and intracellular domain (ICD) between TM3 and TM4

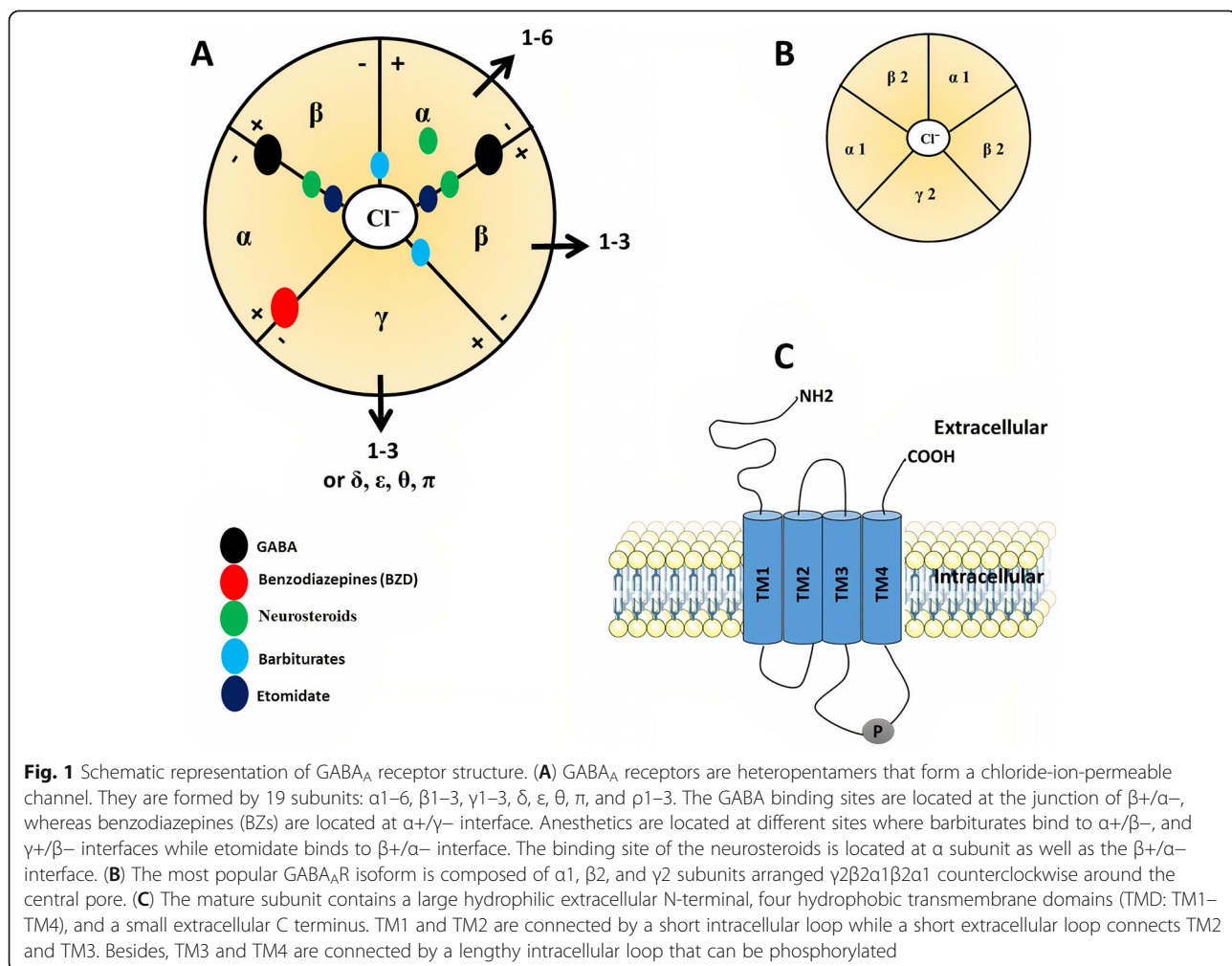


Table 1 GABA_A receptor subunits

Receptor subunit	Gene	Chromosome	Location	Reference
GABA-A alpha 1 ($\alpha 1$)	GABRA1	5	5q34	Gene ID: 2554
GABA-A alpha 2 ($\alpha 2$)	GABRA2	4	4p12	Gene ID: 2555
GABA-A alpha 3 ($\alpha 3$)	GABRA3	X	Xq28	Gene ID: 2556
GABA-A alpha 4 ($\alpha 4$)	GABRA4	4	4p12	Gene ID: 2557
GABA-A alpha 5 ($\alpha 5$)	GABRA5	15	15q12	Gene ID: 2558
GABA-A alpha 6 ($\alpha 6$)	GABRA6	5	5q34	Gene ID: 2559
GABA-A beta 1 ($\beta 1$)	GABRB1	4	4p12	Gene ID: 2560
GABA-A beta 2 ($\beta 2$)	GABRB2	5	5q34	Gene ID: 2561
GABA-A beta 3 ($\beta 3$)	GABRB3	15	15q12	Gene ID: 2562
GABA-A gamma 1 ($\gamma 1$)	GABRG1	4	4p12	Gene ID: 2565
GABA-A gamma 2 ($\gamma 2$)	GABRG2	5	5q34	Gene ID: 2566
GABA-A gamma 3 ($\gamma 3$)	GABRG3	15	15q12	Gene ID: 2567
GABA-A delta (δ)	GABRD	1	1p36.33	Gene ID: 2563
GABA-A epsilon (ϵ)	GABRE	X	Xq28	Gene ID: 2564
GABA-A pi (π)	GABRP	5	5q35.1	Gene ID: 2568
GABA-A theta (θ)	GABRQ	X	Xq28	Gene ID: 55879
GABA-A rho 1 ($\rho 1$)	GABRR1	6	6q15	Gene ID: 2569
GABA-A rho 2 ($\rho 2$)	GABRR2	6	6q15	Gene ID: 2570
GABA-A rho 3 ($\rho 3$)	GABRR3	3	3q11.2	Gene ID: 200959

Data are compiled from NCBI-Gene

which is the site of protein interactions and post-translational modifications that modulate receptor activity (Fig. 1C) [20, 21]. The neurotransmitter GABA, as well as psychotropic drugs such as benzodiazepines (BZDs), bind to the N-terminal at binding sites α - β and α - γ interfaces, respectively. Neurosteroids and anesthetics like barbiturates, on the other hand, are found within the TMD of α and β subunits (Fig. 1A) [22–25].

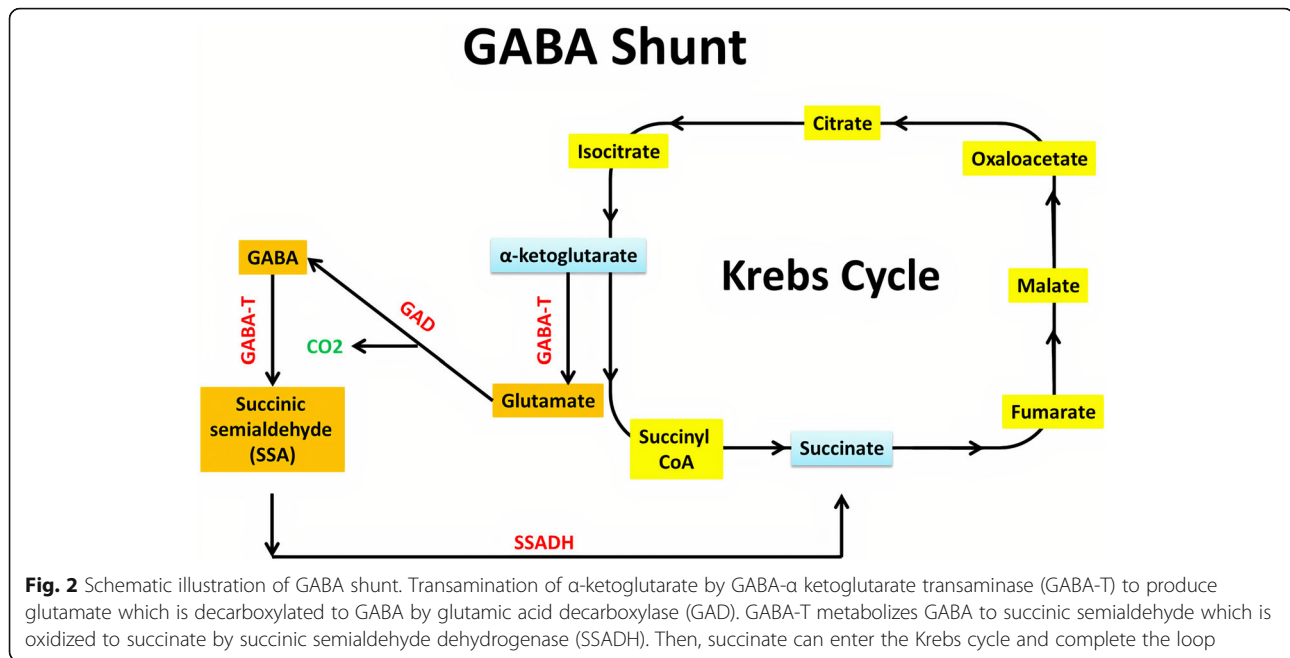
GABA_ARs distribution

In the CNS, some GABA_AR subunits possess broad expression while other subunits exhibit restricted expression. For example, the $\alpha 6$ subunit is expressed only in the cerebellum while the ρ subunit is expressed mainly, but not exclusively, in the retina [26]. GABA_A receptors localized to postsynaptic sites in the brain are mainly composed of the $\alpha 1$ –3, $\beta 1$ –3, and $\gamma 2$ where GABA neurotransmitter can bind with and open chloride channels, thus increasing the anion conductance for a short period (milliseconds), leading to hyperpolarization of a depolarized membrane. This type of GABA inhibition has been termed phasic inhibition. On the other hand, GABA_A receptors composed of the $\alpha 4$ –6, $\beta 2/3$ and δ subunits can localize to extrasynaptic sites where the low GABA concentration can open these receptors for a longer period which is called tonic inhibition [27]. The most popular isoforms of extrasynaptic GABA_ARs mediating tonic inhibition are $\alpha 4\beta\delta$ receptors in the

forebrain, $\alpha 6\beta\delta$ receptors in the cerebellum and $\alpha 1\beta\delta$ receptors in the hippocampus [28]. It has been found that $\alpha 2$, $\alpha 3$, and $\beta 3$ subunit-containing receptors are ~100 times more concentrated at synapses than in the extrasynaptic membrane [29]. Not all $\gamma 2$ -containing receptors are concentrated postsynaptic for example, $\alpha 5\beta\gamma 2$ receptors are found at extrasynaptic sites involved in tonic inhibition [28]. Apart from phasic and tonic inhibition, the $\gamma 2$ subunit is essential for postsynaptic clustering of GABA_A receptors [30] and the $\gamma 3$ subunit substitutes $\gamma 2$ to contribute to the development of the postnatal brain [31]. On the other hand, outside the CNS, GABA_A receptors have been found in different types of immune cells [32, 33], liver cells [34], pancreatic islet β -cells [35], and airway smooth muscle [36]. Despite these observations, the laws that regulate GABA_ARs assembly, as well as the exact process by which GABA_AR isoforms are distributed, remain unknown.

GABA neurotransmission

In 1950, Eugene Roberts and Sam Frankel discovered the major inhibitory neurotransmitter in the CNS of mammals, GABA [37]. Glucose is the main precursor for GABA synthesis, even though other amino acids and pyruvate act as precursors. The GABA shunt is a closed-loop system that produces and conserves GABA (Fig. 2). In GABA shunt, the first step is transamination of α -ketoglutarate produced from the metabolism of glucose



in the Krebs cycle, by GABA- α ketoglutarate transaminase (GABA-T) to produce l-glutamic acid. Glutamic acid is decarboxylated to GABA by glutamic acid decarboxylase (GAD). GAD is an enzyme that uses vitamin B6 (pyridoxine) as a cofactor and is only expressed in cells that use GABA as a neurotransmitter. GABA-T metabolizes GABA to succinic semialdehyde. This transamination happens when α -ketoglutarate is present, it accepts the amino group extracted from GABA, and reforms glutamic acid. Succinic semialdehyde dehydrogenase (SSADH) oxidizes succinic semialdehyde to succinate. It can enter the Krebs cycle, thereby completing the loop [38]. A vesicular transporter helps to package newly synthesized GABA into synaptic vesicles. SNARE complexes help dock the vesicles into the plasma membrane of the cell [39]. Presynaptic neuron depolarisation releases GABA to the synaptic cleft and diffuses toward postsynaptic receptors. It can bind to post-synaptic GABA receptors (GABA_A and GABA_B), which modulate ion channels, hyperpolarize the cell, and prevent action potential transmission. Regardless of binding to GABA_A or GABA_B receptors, GABA serves as an inhibitor. In the case of GABA_A ionotropic receptor, the presence of GABA increases chloride ion conductance into the cell. Consequently, the increased chloride ion influx results in membrane hyperpolarization, and neuronal excitability is reduced [40]. GABA can then be passed into three pathways. The first one is that GABA can be degraded extracellularly by GABA-T into succinate semialdehyde which then enters the citric acid cycle. The second is that the GABA can be reuptaken to nerve terminals for utilization again. The third one is that the GABA can be

reuptaken to the glial cell where it undergoes metabolism to succinic semialdehyde by GABA-T or it becomes glutamine which is transported to neurones, where it is converted to glutamate by glutaminase and re-enters GABA shunt. In glia, GABA cannot be synthesized again from glutamate due to the absence of GAD [41, 42].

The physiological role of GABA and GABA_A receptors

Certainly, GABA/GABA_ARs signaling is the most prominent inhibitory pathway in the CNS. As we discussed before, there are two forms of GABA inhibition: phasic and tonic inhibition. The transient stimulation of GABA_A receptors by GABA reduces postsynaptic neuron excitability, resulting in phasic inhibition [43, 44]. Tonic inhibition, on the other hand, is thought to be a continuous mechanism of inhibition that regulates excitation through long-term hyperpolarization [45]. Tonic inhibition plays an important role in synaptic plasticity, neurogenesis [46, 47] as well as cognitive functions [48, 49]. Any disturbance in phasic or tonic inhibition is associated with many neurological and psychiatric diseases. Thus, modulating these signals has become the basis of drug therapy as well as anesthesia [50–55].

Furthermore, the GABA_A receptor plays a pivotal role in neuronal cell proliferation and fate determination. A pioneering study showed that depolarizing GABA actions leads to a decrease in both DNA synthesis and the number of bromodeoxyuridine (BrdU)-labeled cells at the subventricular zone (SVZ) that mean GABA can affect the proliferation of progenitor cells in rat

embryonic neocortex [56]. Furthermore, GABA or muscimol, a GABA_A receptor agonist, also triggers membrane depolarization and induces proliferation of postnatal cerebellar granule progenitor cells in the developing rat cerebellum [57]. In the adult hippocampus, the neuronal progenitor cells at the subgranular zone (SGZ) show tonic GABAergic conductance. Impairment of this conductivity, as well as the increase in newly generated cells labeled by BrdU, was induced by genetic deletion of GABA_ARs containing $\alpha 4$, but not δ subunits [47, 58, 59]. In the postnatal subventricular zone (SVZ), GABA limits the proliferation of glial fibrillary acidic protein (GFAP)-expressing progenitors thought to be stem cells (also called Type 1 cells) [60]. Also, a recent study suggested that GABA_A receptor contributes to determining the cell fate of neural stem cells [61]. These results indicate that adult neurogenesis may be influenced by multiple functions of GABA_A receptors as well as ambient GABA released in an autocrine/paracrine manner [62, 63].

Of note, GABA_A receptors have additional physiological functions in tissues and organs outside the nervous system [64]. Such as in the pancreatic islet, β -cells synthesize huge amounts of GABA [35]. Via GABA_A receptors, GABA suppresses glucagon secreted by α -cells [65], and increases insulin secreted by β -cells [66]. In addition, GABA stimulates β -cells proliferation and growth [66, 67]. Therefore, targeting GABA/GABA_A signaling is likely to be a part of diabetes treatment [68].

Molecular pharmacology of GABA_A receptors

Apart from GABA, a variety of ligands have been discovered that bind to various locations on the GABA_AR and regulate it. Binding sites are located at particular receptor subtypes, and these subtypes determine the receptors' distinct pharmacological fingerprints [69]. The GABA-binding site, also known as the active site or orthosteric site, is where orthosteric agonists and antagonists bind. Orthosteric agonists, such as GABA, gaboxadol, isoguvacine, muscimol, and progabide [70–72], activate the receptor, resulting in increased Cl⁻ conductance. By contrast, orthosteric antagonists, such as bicuculline and gabazine [73], compete with GABA for binding, inhibiting its effect and lowering Cl⁻ conductance. Allosteric modulators, on the other hand, bind elsewhere on the receptor and exert their effect by causing conformational changes in the receptor either positively (PAM) such as barbiturates, benzodiazepines, z-drugs (nonbenzodiazepines) alcohol (ethanol), etomidate, glutethimide, anesthetics, and certain neurosteroids, or negatively (NAM) such as pregnenolone sulfate and zinc [54, 74, 75]. Non-competitive chloride channel blockers (ex., picrotoxin) are ligands that bind to or near the central pore of the GABA_AR and block Cl⁻ conductance [76]. Moreover, silent allosteric modulators (SAM)

are a class of GABA_AR modulators that can compete with a PAM or a NAM for the occupation of the binding site such as flumazenil [75, 77]. The characteristics of ligands that contribute to receptor activation are usually used as anxiolytic, anticonvulsant, sedative, and muscle relaxant drugs. On the other side, ligands that inhibit receptor function usually have opposite pharmacological effects such as convulsion and angiogenesis [78, 79]. Interestingly, some subtypes of NAM (ex., $\alpha 5$ IA) are being studied for their nootropic properties as well as potential therapies for GABAergic medication adverse effects [80].

GABA and GABA analogs

Cys-loop receptors typically have their neurotransmitter binding site at the extracellular interface between two neighboring subunits. The binding site's principal face (+) is made up of three loops (A, B, and C), whereas the complementary face (-) comprises three β -strands and one loop (D, E, F, or G) [81, 82]. In GABA_ARs, $\alpha\beta\gamma$ subtype (2 α :2 β :1 γ) has two GABA binding sites at the $\beta +/\alpha -$ interfaces (Fig. 1A). When GABA occupies just one site, the channel opens; however, when both sites are occupied, the chances of channel opening rise dramatically [83]. Besides, chemicals with similar structures to GABA can attach to GABA binding sites and give different effects such as muscimol (agonist), gaboxadol (partial agonist), and bicuculline (competitive antagonist) [82].

Actually, it is still a mystery how amino acid residues interact with GABA. However, in a previous study based on $\alpha\beta\gamma$ subtype, GABA formed hydrogen bonds with $\alpha 1$ T129 and $\beta 2$ T202, salt bridges with $\alpha 1$ R66 and $\beta 2$ E155, and cation- π interaction with $\beta 2$ Y205 [84]. On the other hand, $\beta +/\alpha -$ interface has aromatic residues formed by β Y97, β Y157, β F200, β Y205, and α F64 which are conserved at the $\beta +/\beta -$, $\beta +/\gamma -$, and $\beta +/\delta -$ interfaces. Furthermore, the GABA-binding subunit residues R131, T129, and L127 are maintained at the equivalent places in the β , γ , and δ subunits [81, 84, 85]. Future studies will examine whether GABA and other structurally similar chemicals are attracted to these non-canonical sites, as well as how these sites may influence receptor activation.

Benzodiazepines

Benzodiazepines (BZDs) are commonly used in different treatments related to anxiety, sleep disorders, seizure disorders, muscle spasms, and some forms of depression [86]. BZD allosterically modulate GABA_AR and give its therapeutic effect through binding to the $\alpha +/\gamma -$ interface (Fig. 1A) and increasing Cl⁻ conductance [24, 87]. Interestingly, amino acids involved in the binding sites of BZDs are homologous to that of the GABA binding site at the $\beta +/\alpha -$ interface [88]. Besides, mutations that

converted histidine to arginine ($\alpha 1H101R$, $\alpha 2H101R$, $\alpha 3H126R$, and $\alpha 5H105R$) at the $\beta 2\gamma 2$ subtype of $GABA_A$ Rs eliminated diazepam activity, while reverse mutations (from R to H) elicited the diazepam response [89]. BZD-sensitive $GABA_A$ Rs subtypes are formed of two α subunits with two β subunits and a γ subunit (Fig. 1A) [90]. Likewise, $GABA_A$ R containing $\alpha 4$, $\alpha 6$, and $\gamma 2$ subunits, potently bind many BZD ligands [91, 92]. But subtypes containing δ are relative with low abundance, and the subunits replacing γ and δ , such as ϵ , are even rarer [93]. Of note, the $GABA_A$ R subtypes containing δ subunits are located extrasynaptically inducing tonic inhibitory currents in major cell populations including cerebellar and hippocampal granule cells [43, 93]. It was thought that these subtypes are not capable to bind any BZD ligands, lacking the high-affinity $\alpha +/\gamma -$ (site 1), but later it was found to bind some BZD ligands with lower affinity at distinct other sites on the $GABA_A$ R [54].

Benzodiazepines as zolpidem (an imidazopyridine) and other clinically used hypnotics like zaleplon (a pyrazolopyrimidine) and zopiclone (a cyclopyrrolone), as well as quinolones, triazolopyridazines, and beta-carbolines show a higher affinity for $\alpha 1$ -containing receptors than for $\alpha 2$ - or $\alpha 3$ -containing subtypes, while they do not affect $\alpha 5$ -containing $GABA_A$ Rs [93, 94]. Also, imidazobenzodiazepine oxazole derivatives have shown some $\alpha 2/\alpha 3$ selectivity [95]. Pyrazoloquinolinones, which are examples for BZD site-active PAM in γ -containing subtypes, demonstrate a wide range of effects as well as selectivity for α and β subunits [54]. Also, BZD-site ligands have more or less efficacy than traditional BZD agonists on the traditional BZD-sensitive subtypes, and unexpected efficacy on the diazepam-insensitive subtypes like $GABA_A$ R containing $\alpha 4$ or $\alpha 6$, or α and β without γ [96, 97].

Alpha51A is selective inverse agonists that bind to the BZD site at the $\alpha 5$ subtype that is highly expressed in the CA1 region of the hippocampus. It has been suggested to improve cognitive functions [98]. Such $\alpha 5$ inverse agonists also reduce side effects of BZDs, general anesthetics [99], and alcohol [100]. They may be useful for treating Down syndrome, autism spectrum disorder, schizophrenia, and affective disorders [101].

Anesthetics

$GABA_A$ Rs are remarkable targets of variable volatile anesthetics, intravenous anesthetics, etomidate, and propofol, as well as steroid anesthetics, barbiturates, and ethanol [102]. Anesthetic binding sites on the $GABA_A$ R can be identified using site-directed mutagenesis [103], substituted cysteine modification protection (SCAMP) [104], or photo-affinity labeling [102, 105]. At higher concentrations, some anesthetics, especially the intravenous anesthetics, etomidate, propofol, and barbiturates, could

directly activate $GABA_A$ Rs in the absence of GABA. Such direct activation distinguished them as GABA-mimetic from benzodiazepines which lack this property. Studies that were based on site-directed mutagenesis produced several residues of interest, particularly in the transmembrane regions of the α and β subunits, for both volatile and intravenous anesthetics [106].

Of note, methionine residues, especially $\alpha M236$ and $\beta M286$ located in the M1 and M3 domains respectively, have been shown to be significant determinants of etomidate binding and function in experiments that used mutagenesis and photoreactive etomidate analogs. Based on crystal structures of $GABA_A$ Rs, $\alpha M236$ and $\beta M286$ are expected to be found at the $\beta +/\alpha -$ interfaces in the TMD, below the GABA binding sites (Fig. 1A). Also, $\alpha T237$ (M1), $\alpha I239$ (M1), $\alpha L232$ (M1), $\beta V290$ (M3), and $\beta F289$ (M3) are among the additional residues linked to etomidate binding and function [107, 108]. Besides, in $\alpha 1\beta 3\gamma 2$ $GABA_A$ Rs, other anesthetic binding sites including $\alpha +/\beta -$ and $\gamma +/\beta -$ interfaces (Fig. 1A) have been identified using photoreactive analogs of barbiturate where $\alpha A291$ (M3), $\alpha Y294$ (M3), $\beta M227$ (M1), and $\gamma S301$ (M3) were among the binding residues [82, 109]. Moreover, in the TMD of $\beta 3$ homomeric $GABA_A$ Rs at $\beta +/\beta -$ interface, photoreactive propofol can bind to $\beta (+)$ M286, $\beta (+)$ F289, and $\beta (-)$ M227 residues inducing functional activity of the receptor [110–112].

It has been found that $\beta 2$ and $\beta 3$ subunits were significant for modulation of $GABA_A$ R by i.v. anesthetics. In addition, transgenic mice that were generated through $\beta 2$ (N265S) and $\beta 3$ (N265M) mutations in the $GABA_A$ R became insensitive to the actions of propofol and etomidate [113, 114]. The affinity and efficacy of barbiturate depend on the composition of the subunit, but the α subunit seems to be more important than β [115]. Recently, it has been suggested that the binding of barbiturate, etomidate, and propofol is predominantly at the $\alpha \beta +/\alpha -\gamma$ interface as well as the $\alpha +/\beta -$ or $\alpha +/\gamma -$ TMD interfaces in $\alpha 1\beta 2\gamma 2$ [69, 116]. Other photo-affinity labeling depending studies suggested that binding sites for barbiturates and etomidate at $\alpha 4\beta 3\delta$ $GABA_A$ R subtypes at the $\beta +/\alpha -$, and $\beta +/\beta -$ TMD interfaces, respectively, were not suitable for binding of delta selective compound 2 (DS2) or alphaxalone [117].

Neurosteroid

Endogenous steroids exhibit $GABA_A$ R-mediated neuroactive effects including anesthesia, anticonvulsant, analgesia, and sedation. The most common examples are allopregnanolone and its synthetic analogs [118]. Although the exact position of the neurosteroid binding sites has yet to be determined, many residues in the TMDs have been shown to impact neurosteroid activity, such as $\alpha S240$ (M1), $\alpha Q241$ (M1), $\alpha N407$ (M4), $\alpha Y410$ (M4), $\alpha T236$

(M1), and β Y284 (M3) [119–121]. The modulatory and activation sites are located at the TMDs of α subunit and $\beta +/\alpha -$ interfaces respectively (Fig. 1A) [82, 122].

Flavonoids

Flavonoids are present in most plants and a few microorganisms. They have been discovered as modulators of the BZD-site of GABA_ARs, but the variability of compounds within this group participated in showing their potential action at more than one additional binding site on GABA_ARs. Flavonoids can act as either negative, positive, or neutralizing on GABA_ARs or directly as allosteric agonists [123]. Flavonoids share the elementary structure of a phenylbenzopyran, most commonly of a flavan (2-phenylchromane). Subgroups contain isoflavones, flavonoles, flavones, flavanonole, flavanones, and flavanoles. Among these groups, isoflavones and flavones particularly have been found to interact with the binding site of BZD [124]. Structure-activity experiments have illustrated that flavones have higher potency on BZD radioligand binding than their flavanone or flavanol counterparts. Besides, glycosylation had a negative influence on binding [125]. Flavonoids can also interact with flumazenil-sensitive or -insensitive GABA_ARs [123]. Some of the flavonoids have shown subtype-selectivity like flavan-3-ol ester Fa131 [126] or 6,2'-dihydroxyflavone [127]. The flavone hispidulin showed potent activity in crossing the blood-brain barrier associated with the $\alpha 6\beta 2\gamma 2$ subtype of GABA_ARs, which is used to reduce the susceptibility of seizures [128].

Cannabinoids

Cannabinoids are chemical substances present in the cannabis plant. The phytocannabinoid tetrahydrocannabinol (THC) is the primary psychoactive compound in cannabis. Besides, cannabidiol (CBD) is another significant component of the plant [129]. It has been found that CBD has sedative, anxiolytic, and anticonvulsant effects and has been suggested for treating pediatric epilepsies such as Dravet syndrome [130]. CBD, also, showed a low affinity for the main cannabinoid receptor and exhibits an activity profile similar to that of GABA PAMs inducing anxiolytic and anticonvulsant effects [131].

Endocannabinoids, such as 2-Arachidonoylglycerol (2-AG), 2-Arachidonyl glyceryl ether, N-Arachidonoyl dopamine (NADA), Arachidonylethanolamine (AEA), and Lysophosphatidylinositol (LPI) [132], are substances produced in the body activating cannabinoid receptors (CB1, CB2) [133, 134]. Additionally, they have been identified as positive modulators for GABA_AR subtypes [135]. Studies on recombinant receptors showed that 2-AG increases GABA_AR activity at low non-saturating GABA concentrations while decreasing the activity at

high saturating GABA concentrations. Therefore, the impact of endocannabinoids on GABA_AR depends on the regulation of GABA inhibition [136].

Picrotoxin

Picrotoxin is a plant-derived product, with a universal efficacy as GABA_AR's chloride channel blocker. Picrotoxin is found naturally in the *Anamirta Cocculus* plant, although it can be synthesized chemically [137, 138]. It has been utilized as a CNS stimulant, and antidote for poisoning by CNS depressants and barbiturates [139]. However, due to the toxicity of picrotoxin, it is currently used only in research. Furthermore, numerous studies indicated that a wide range of molecules from various chemical families had an affinity for picrotoxin-binding sites such as *t*-butylbicyclophosphorothionate (TBPS), *t*-butylbicycloorthoobenzoate (TBOB), pentylenetetrazole, and some insecticides (ex., dieldrin and lindane) [140–142]. A study by Othman et al. (2012) [143] found that low concentrations of GABA increase picrotoxin and TBPS binding affinity to GABA_AR containing $\alpha 1\beta 2\gamma 2$, while application of GABA at high concentration reduces their binding affinity to the receptor reducing channel blocking activity. This indicates that picrotoxin and ligands of picrotoxin-binding sites are highly dependent on the regulation of GABA inhibition.

Pharmacology of δ -containing GABA_ARs

The unique role of the δ subunit in extra-synaptic GABA_ARs, a group of receptors responsible for tonic GABAergic inhibition has generated immense therapeutic and research interests. However, the complicated properties of the δ subunit assembly and the rarity of δ -selective ligands are the main reasons hindering progress in pharmacological studies of these receptors. Variable compounds have been claimed to be selective for the δ subunit. The hypnotic drug THIP (4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridin-3-ol) and gaboxadol are examples of compounds that are known by their direct activation of $\alpha\beta\delta$ with higher efficacy and potency than $\alpha\beta\gamma$ but does not discriminate between $\alpha\beta$ and $\alpha\beta\delta$ receptors [144, 145]. Similar to THIP, anesthetics, as well as neurosteroids, also show more pronounced action at δ -containing GABA_ARs, but their activity is independent of subunit composition, these compounds are not considered to be δ -selective. In contrast, 4-chloro-N-(2-thiophen-2-ylimidazo[1,2-a]pyridin-3-yl) benzamide which was found to be a positive modulator at $\alpha 4/6\beta\delta$, has limited efficacy at $\alpha\beta\gamma$ and is inactive at $\alpha\beta$ GABA_ARs [146].

GABA_A receptor dysfunction and neuro-psychiatric disorders

Epilepsy

Epilepsy is a neurological disease characterized by frequent and unexpected seizures caused by abnormal

brain electricity, which results in loss of consciousness and unusual behaviors [147]. Around 65 million people are affected worldwide, of all ages and genders [148]. An imbalance between excitation and inhibition induced by impaired GABAergic signaling can trigger various forms of epilepsy [149, 150]. Several studies have demonstrated the importance of GABA_A receptors as targets for anti-epileptic drugs [45, 151, 152]. Mutations in GABA_A receptor subunit genes have been linked to several types of idiopathic epilepsy in which the pathophysiological consequences of the mutations are impairments in the gating characteristics of the channel or receptor trafficking [4]. The severity of the disorder appears to depend on the type of mutation (nonsense, missense, or frameshift), its location in the gene (promoter or protein-coding region), the affected region of the encoded protein (intra-/extracellular or transmembrane) and the affected subunit gene [4]. Some mutations in genes encoding the $\alpha 1$, $\alpha 6$, $\beta 2$, $\beta 3$, $\gamma 2$, or δ subunits of GABA_ARs have been detected in both animal models of epilepsy and patients with epilepsy [153, 154]. Likewise, Dravet syndrome, also known as severe myoclonic epilepsy in infancy (SMEI), is a form of epilepsy that affects children at the age of approximately 1 year as a result of mutations in genes encoding the $\alpha 1$, $\beta 1$, $\beta 2$, and $\gamma 2$ subunits of GABA_ARs [4, 155]. Of note, several GABA_AR mutations associated with epilepsy lead to abnormal trafficking of the receptors and thus partially or completely impair their expression on the synaptic plasma membrane [155, 156]. Likewise, a study by Dejanovic et al. [157] discovered a missense mutation in GPHN gene, the gene encoding the gephyrin protein, in a patient with Dravet syndrome. Gephyrin is the main protein that clusters and stabilizes GABA_ARs at the inhibitory postsynaptic membranes of the central nervous system [158]. Moreover, during the epileptogenic period, expression of the gephyrin protein decreases gradually in the neocortex before returning to baseline during the chronic phase [159]. These findings suggest that the downregulation of GABA_AR subunits or their interactors that play a functional role in receptor activity, such as gephyrin, maybe the origin of the disease and thus could be used as drug targets.

Alzheimer's disease

Alzheimer's disease (AD) is one of the primary diseases that cause neurodegeneration. Clinically, AD is marked by significant cognitive deficits and regarded as the most common cause of dementia. The aggregation of misfolded amyloid-beta (A β) protein, which forms amyloid plaques in the gray matter of the brain, is the origin of AD pathophysiology. Amyloid plaques, neuronal dysfunction, and tangles of neural fibers are major pathological features of the disease [160, 161]. Several experiments, in both AD patients and mice, have shown

that accumulation of misfolded A β interferes with GABAergic interneuron activity, causing impaired synaptic communication and loss of neural network activity, which eventually leads to cognitive dysfunction [162–165]. A recent study showed transcriptional downregulation of $\alpha 1$, $\alpha 2$, $\alpha 3$, $\alpha 5$, $\beta 1$, $\beta 2$, $\beta 3$, δ , $\gamma 2$, $\gamma 3$, and θ subunits of GABA_A receptors, and GAD enzyme in the middle temporal gyrus (MTG) of post-mortem brain samples from AD patients. These alterations impair the balance between excitatory and inhibitory pathways that may lead to cognitive dysfunction in AD [166]. Likewise, in biochemical studies, GABA neurotransmitter levels were substantially lower in the CSF as well as the temporal cortex of Alzheimer's patients, implying impaired synaptic activity and neuronal transmission [44, 167–169]. Also, a study by Limon et al. [170] showed that most aspects of the GABA system were impaired in the brains of AD patients, such as GABAergic neural circuit, GABA levels, and expression levels of GABA_A receptors. Furthermore, in AD mice, activating GABA_A receptors with baicalein (positive allosteric modulator of the benzodiazepine site of the GABA_AR) for 8 weeks significantly reduced A β production, improved cognitive function, and decreased pathological features [171]. As a result, GABA_A receptors seem to be a potential therapeutic target in the treatment of AD.

Cervical dystonia

Cervical dystonia (CD) is the most frequent type of adult-onset focal dystonia. It is a neurological disorder marked by involuntary and prolonged muscle contractions that cause irregular postures and neck tremors [172–174]. Studying the pathophysiology of isolated cervical dystonia using different methods such as magnetic resonance spectroscopy (MRS), positron emission tomography (PET), and functional magnetic resonance imaging (f-MRI) demonstrated an alteration in the GABA-mediated inhibitory signaling pathway in the cortical, cerebellar, and basal ganglia regions of the brain [175]. Similarly, a significant number of functional defects have been identified in the thalamus of patients with CD [176], and blocking GABA_A receptors in the thalamus triggered CD-like symptoms in monkeys [177]. According to a recent study, GABA levels in the right thalamus were decreased in a sample of adult-onset CD patients, and the availability of GABA_A receptors was negatively correlated with disease duration and the severity of dystonia [178].

Brain injury

Several studies investigated whether GABA signaling pathways are involved in several forms of brain injuries using different stroke mice models. As reported in earlier studies, increasing GABA inhibition has shown a

neuroprotective role at stroke onset. In contrast, increased GABAergic tonic inhibition at extrasynaptic GABA_A receptors would adversely affect and exacerbate stroke pathology. Also, these findings were in line with study results obtained from knockout mice models lacking either $\alpha 5$ -GABA_A or δ -GABA_A receptors, which have revealed better recovery from stroke than healthy mice models because of GABAergic signaling remission [179, 180].

Autism spectrum disorder

Autism spectrum disorder (ASD) has three characteristic behavioral features: impaired communication and social deficits, and repetitive behaviors. Several studies concluded an imbalance in the glutamatergic/GABAergic signaling pathways and neuroinflammation process were associated with ASD pathophysiology and were also detected in several ASD mice models [181]. Earlier studies reported the presence of molecular-level cortical abnormalities related to GABAergic signaling dysfunction in the brains of ASD. The excitatory and inhibitory signaling imbalance caused by variations in GABA levels represents one of the characteristic features behind behavioral deficits in autism [182]. Mendez et al. [183] conducted a PET imaging study using a radioactive ligand [¹¹C]-Ro15-4513 VT for tracing levels of GABA_A receptor $\alpha 5$ subunits in ASD. The results showed a reduction in GABA_A receptors in the brain's two limbic areas (amygdala and nucleus accumbens) of autism patients. Contrary to previous findings, a recent study demonstrated that the impairment in the GABAergic system in ASD mouse models and autistic patients was not associated with alterations in GABA receptor numbers between healthy and ASD controls, as concluded by an earlier study [184]. Also, a recent meta-analysis was conducted to verify earlier findings supporting the association between different genetic variants of GABA_A receptor subunits and the risk of developing autism in children. In conclusion, the study showed no association between GABA receptor subunits ($\beta 3$, $\alpha 5$, and $\alpha 3$) and child autism [185].

Schizophrenia

Schizophrenia is a multifactorial major psychiatric disorder whose etiology has been associated with hundreds of protein-coding genes reported by different genome-wide association studies. Changes in post-translational modifications of various proteins including GABA_A receptors and their contribution to schizophrenia pathophysiology were reported [186]. A previous study showed glycosylation changes in multiple protein receptor subunits in the brains of schizophrenic patients, such as AMPA and GABA_A receptor subunits [187].

Specifically, several post-mortem brain studies conducted using lectin affinity analysis and enzyme deglycosylation of GABA_A receptors of superior temporal gyrus of schizophrenic brains demonstrated a decrease in high-mannose N-glycans residues of GABA-associated proteins in individuals with schizophrenia that were specific to different GABA_A receptor subunits on the $\alpha 1$, $\alpha 4$, $\beta 1$, $\beta 2$, and $\beta 3$ subunits; increased high-mannose N-glycans on $\beta 1$ subunit; decreased high-mannose N-glycans on $\alpha 1$ subunit; altered total N-glycans on $\beta 2$ subunits. These N-glycosylation alterations were further associated with abnormal trafficking and localization of $\beta 1$ / $\beta 2$ subunits leading to an aberrant inhibitory signaling system observed in schizophrenia [188, 189].

Furthermore, Marques and his co-workers [190] investigated the availability of $\alpha 5$ -GABA_A receptors in the hippocampus using PET imaging for hippocampal regions schizophrenic and healthy controls. The study results demonstrated a reduction of [¹¹C]-Ro15-4513 VT ([¹¹C]-Ro15-4513), which is a radioactive tracer used by PET scans to assess the total volume of distribution for $\alpha 5$ -GABA_A receptors in the hippocampus of untreated schizophrenic patients versus healthy controls. In contrast, there were no differences between healthy control and the second cohort of patients treated with antipsychotics. These findings were also positively correlated with scaling using PANSS (Positive and Negative Syndrome Scale) scores (i.e., is a medical scale system that measures the severity of schizophrenic symptoms).

Depression

Major depression is one of the debilitating diseases that leads to neurons' anatomical and functional changes in the brain's prefrontal cortex and is induced by chronic stress. Earlier studies had concluded that dysfunction in monoaminergic signaling was the main contribution to depression pathophysiology. Lately, accumulating evidence has suggested the potential role of GABAergic signaling dysfunction in predispositions of depression as it has been reported that both depression and chronic stress are associated with an imbalance in inhibition, and excitation of neuronal signaling resulted from a deficiency in neuronal transmission onto the brain's prefrontal cortex (PFC). This imbalance resulted from the deficient transmission of GABAergic inhibitory signals onto the brain's excitatory glutamate interneurons. In this context, several studies were conducted to demonstrate the correlation between GABAergic dysfunction and depression. For instance, a study showed using magnetic resonance imaging established decreased GABA and GAD67 levels and alterations in distinct types of GABA receptor subunits in the brains of depressed patients and stressed mice models. Studies conducted on

genetically modified depressed mice models lacking specific GABA receptors showed depressive mice behaviors [191].

Data from magnetic resonance imaging MRI studies reported a reduction in hippocampal volume of the brain of depressed patients, which leads to alterations in neural circuits of different areas of the brain related to emotionality, such as amygdala and prefrontal cortex. Interestingly, study results using depressed mice models lacking GABA_A receptors showed that any alterations in the brain's GABAergic system were presented by cognitive, neuroanatomical, and behavioral deficits like significant depression disorder symptoms presented by depressed animal models. Accordingly, it is now presumed that the GABAergic system plays a vital role in controlling neuronal transmission in neuronal maturation in the hippocampus. Therefore, it is considered a therapeutic target for potential antidepressant drugs [28, 192].

Attention and social behavior

Several studies have shown that inhibiting cortical GABA_A receptors causes impaired attention [16, 193–197], social behavior [198], and decision-making [199]. Recently, it has been demonstrated that mice models having impaired 5-alpha GABA_A receptors were presented with behavioral deficits like symptoms associated with attention and social disorders [194].

Conclusion

Deep insights into the different GABA_A receptor isoforms' composition, arrangement, subunit interactors, and molecular pharmacology will give us a clear vision to understand alterations that may lead to CNS disorders. In our view, these discussions are of vital importance in drug discovery and development in the future.

Abbreviations

2-AG: 2-Arachidonoylglycerol; AD: Alzheimer's disease; AEA: Arachidonylethanolamine; ASD: Autism spectrum disorder; Aβ: Amyloid beta; BrdU: Bromodeoxyuridine; BZ/BZD: Benzodiazepine; CB: Cannabinoid receptors; CBD: Cannabidiol; CD: Cervical dystonia; DS2: Delta selective compound 2; f-MRI: Functional magnetic resonance imaging; GABA: γ-Aminobutyric acid; GABAARs: γ-Aminobutyric acid sub-type A receptors; GABA-T: GABA transaminase; GAD: Glutamic acid decarboxylase; GFAP: Glial fibrillary acidic protein; GPCRs: G protein-coupled receptors; ICD: Intracellular domain; LPI: Lysophosphatidylinositol; MRS: Magnetic resonance spectroscopy; MTG: Middle temporal gyrus; NADA: N-Arachidonoyl dopamine; NAM: Negative allosteric modulation; PAM: Positive allosteric modulation; PANSS: Positive and Negative Syndrome Scale; PET: Positron emission tomography; PFC: Prefrontal cortex; SAM: Silent allosteric modulators; SCAMP: Cysteine modification protection; SMEI: Severe myoclonic epilepsy in infancy; SSADH: Succinic semialdehyde dehydrogenase; SVZ: Subventricular zone; THC: Tetrahydrocannabinol; TMD: Transmembrane domains

Acknowledgements

Not applicable.

Authors' contributions

AG conceived the concept, designed the study, and prepared the figures and tables. AG, DA, ASAS, and DEEH collected the literatures from various resources and drafted the manuscript. AG, DA, and DEEH revised and corrected the manuscript. The authors read and approved the final manuscript.

Funding

Not applicable

Availability of data and materials

All data generated or analyzed during this study are included in this manuscript.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Competing interests

Not applicable

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Received: 24 June 2021 Accepted: 8 August 2021

Published online: 21 August 2021

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