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# Tonic GABA$_A$ Receptors as Potential Target for the Treatment of Temporal Lobe Epilepsy

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**Abstract** Tonic GABA$_A$ receptors are a subpopulation of receptors that generate long-lasting inhibition and thereby control network excitability. In recent years, these receptors have been implicated in various neurological and psychiatric disorders, including Parkinson's disease, schizophrenia, and epilepsy. Their distinct subunit composition and function, compared to phasic GABA$_A$ receptors, opens the possibility to specifically modulate network properties. In this review, the role of tonic GABA$_A$ receptors in epilepsy and as potential antiepileptic target will be discussed.

**Keywords** Extrasynaptic GABA$_A$ receptor · Epilepsy · Seizures · Tonic · Antiepileptic drugs

## Abbreviations

| | |
|---|---|
| AED | Antiepileptic drug |
| CA | Cornu ammonis region |
| DG | Hippocampal dentate gyrus |
| GABARAP | γ-Aminobutyric type A receptor-associated protein |
| GABA$_A$R | γ-Aminobutyric type A receptor |
| IPSP | Inhibitory postsynaptic potential |
| PSD | Postsynaptic density |
| TLE | Temporal lobe epilepsy |

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## Introduction

Worldwide, more than 50 million people are suffering from epilepsy [1]. Temporal lobe epilepsy (TLE) is the most common type of partial onset epilepsy [2]. Epilepsy has a severe impact on patients' quality of life, first, because patients experience unpredictable seizures that restrict them in activities of daily living and second, because patients can suffer from several neuropsychiatric comorbidities such as depression or cognitive decline. Current treatment options for epilepsy are insufficient. Approximately 30 % of patients are drug resistant [3], which is defined as failure to achieve seizure freedom despite two tolerated, adequately applied antiepileptic drug (AED) schedules [4]. Furthermore, AEDs can have side effects, including somnolence, behavioral changes, dizziness, and weight gain. Therefore, there is an urgent need for more efficacious AEDs with fewer side effects.

The γ-aminobutyric acid (GABA) type A receptor (GABA$_A$R) is an important target for AEDs, as it is the most important inhibitory receptor in the central nervous system and therefore plays an important role in the development and maintenance of epilepsy. Upon binding of the neurotransmitter GABA to the ionotropic GABA$_A$R, the receptor opens allowing chloride and bicarbonate ions to diffuse into the cell. This results in hyperpolarization and a higher excitation threshold, i.e., an inhibitory postsynaptic potential (IPSP) [5, 6]. GABA$_A$Rs mediate two different types of inhibition:

phasic inhibition characterized by a short-lasting IPSP and tonic inhibition characterized by persistent, long-lasting IPSP. GABA$_A$Rs mediating tonic inhibition are different from those mediating phasic inhibition. They are located outside the synapse and hence are referred to as perisynaptic or extrasynaptic receptors. Moreover, the subunit composition of tonic GABA$_A$Rs differs from that of phasic GABA$_A$Rs.

Due to its long-lasting hyperpolarization, tonic inhibition can be considered as a constant "brake on the system" counterbalancing excitation [7]. Tonic currents are therefore involved in a broad array of vital physiological functions, such as regulating neuronal excitability, network oscillations, synaptic plasticity, neurogenesis, neuronal development, information processing, and cognition [5, 8–14]. The importance of this type of inhibition is further emphasized when realizing that under physiological conditions, the charge carried by tonic GABA currents is bigger than that of synaptic currents. For instance, tonic inhibition is responsible for generating 75 % of the total inhibitory charge received by hippocampal neurons [15].

Considering the important role of tonic GABA signaling in regulating network excitability, alterations in tonic signaling must play a role in epilepsy. The current review discusses physiological tonic GABA signaling, its role in TLE, and describes existing and future strategies using tonic currents as novel targets for antiepileptic treatment.

## Physiology of Tonic GABA Signaling

In order to discuss the pathophysiological changes taking place in epilepsy, it is first necessary to understand the physiological principals governing tonic signaling in the central nervous system. The following paragraphs will discuss the molecular composition of GABA$_A$Rs, determinants influencing tonic signaling including the role of GABA$_B$ receptors (GABA$_B$Rs), the specific role of tonic inhibition in interneurons, and clustering of GABA$_A$Rs.

### Subunit Composition of Tonic GABA$_A$Rs

GABA$_A$Rs are heteropentamers assembled from α1-6, β1-3, γ1-3, δ, ε, π, and θ subunits [16, 17]. In theory, these different subunits can combine into thousands of different GABA$_A$Rs. In reality however, GABA$_A$Rs have preferential configurations, which means that the number of different GABA$_A$Rs expressed in vivo is limited. Mostly, two α subunits combine with two β subunits and either a γ or a δ subunit [18]. Some subunits are inhomogeneously distributed over the brain [19, 20]. For instance, α5 is predominantly found in the hippocampal dentate gyrus (DG) and cornu ammonis (CA) 1 and 3 region. In contrast, δ and γ subunits are found in the cortex, hippocampus, thalamus, striatum, and cerebellum. At the subcellular level, some subunits and subunit combinations are preferentially expressed at extrasynaptic sites (Table 1). For instance, α5 subunits and receptors consisting of an α4, α6, and δ subunit are predominantly expressed at extrasynaptic sites [21, 22], whereas, γ2 is mostly expressed at the synaptic site [7, 9, 21, 23–27]. To date, knowledge of the processes guiding these preferential expression sites is incomplete. In general, it is assumed that guidance is driven by the interaction between subunits, anchoring proteins and the cytoskeleton [28].

In addition to a specific distribution, the subunit composition of GABA$_A$Rs also influences the affinity for GABA. For instance, receptors containing an α3 subunit have a higher affinity for GABA than α5 containing GABA$_A$Rs [29–32]. Similarly, δ subunit containing GABA$_A$Rs have a higher affinity than those expressing a γ subunit. This implicates that extrasynaptic GABA$_A$Rs have a higher affinity for GABA than synaptic ones [5, 33]. Finally, the subunit composition can also affect the kinetic properties of GABA$_A$Rs, e.g., δ subunit containing GABA$_A$Rs desensitizes slower [5].

## Activation of Tonic GABA$_A$Rs

Tonic GABA$_A$Rs can be activated in several ways (Fig. 1). First of all, repetitive activation of the presynapse results in increased levels of GABA in the synaptic cleft. This is due to insufficient time for clearance by GABA transporters (GATs). As a consequence, GABA can accumulate in the synaptic cleft. Consequently, GABA diffuses to the extracellular space where it activates extrasynaptic receptors [21, 34, 35]. The degree and speed of diffusion are influenced by parameters such as the distance between the synaptic cleft and receptor, diffusional barriers, and geometry of the synapse [36].

Second, tonic GABA$_A$Rs may be activated by GABA that is released from non-vesicular sources [37, 38]. Extracellular GABA may come from glial cells or dendrites (for a review, see Yoon and Lee [39]) [40–44], for instance by GAT reversal [45–51].

Third, opening of tonic GABA$_A$Rs can occur spontaneously in the absence of synaptic activation and even in the absence of extracellular GABA [52, 53]. This has been shown in vitro for β1 subunit containing GABA$_A$Rs in the rat [54], the human α1β3ε receptor [55], human α1β1 and α1β1γ2 receptors [56], and in rat hippocampal slices [57].

The three proposed mechanisms of tonic GABA$_A$R activation might be complementary: In case of baseline network activity, tonic activation may be caused by non-vesicular sources of extracellular GABA or by spontaneous opening, while in case of excessive

**Table 1** Subunit composition of extrasynaptic GABA$_A$Rs depends on brain region and cell type

| GABA$_A$R subunits | Structure | Cell type |
| --- | --- | --- |
| α5βγ | Subiculum | Pyramidal cells [16] |
| | | Interneurons [17] |
| | Hippocampus | Granular cells [18] |
| | CA1 | Pyramidal cells [7] |
| α6βδ | Cerebellum | Granular cells [19–21] |
| | Hippocampus | Pyramidal cell [7] |
| α4βδ | Thalamus | Relay neuron [22–26] |
| | Hippocampus | Granular cells [18, 20, 27, 28] |
| | Frontoparietal cortex | Pyramidal cells [29] |
| α1β2δ | Hippocampus (Molecular layer) | Interneurons [25] |
| α3βγ | Basolateral amygdala | Pyramidal cells [30] |
| α5β3γ2 | Striatum | Juvenile D1+/D2+ cells [31, 32] |

network activity, GABA may spill into the extracellular space.

The magnitude of tonic signaling is influenced by several factors, such as the amount of ambient GABA and the availability of receptors. Moreover, neuromodulators can alter tonic GABA signaling. An example of neuromodulators are neurosteroids, a group of neuromodulators that influences δ subunit containing tonic GABA$_A$Rs. Binding of these neurosteroids increases tonic inhibition. Next to this acute effect, neurosteroids also cause downregulation of certain subunits such as the γ2 and δ subunits in the DG [58]. Consequently, tonic and phasic currents decreased [59].
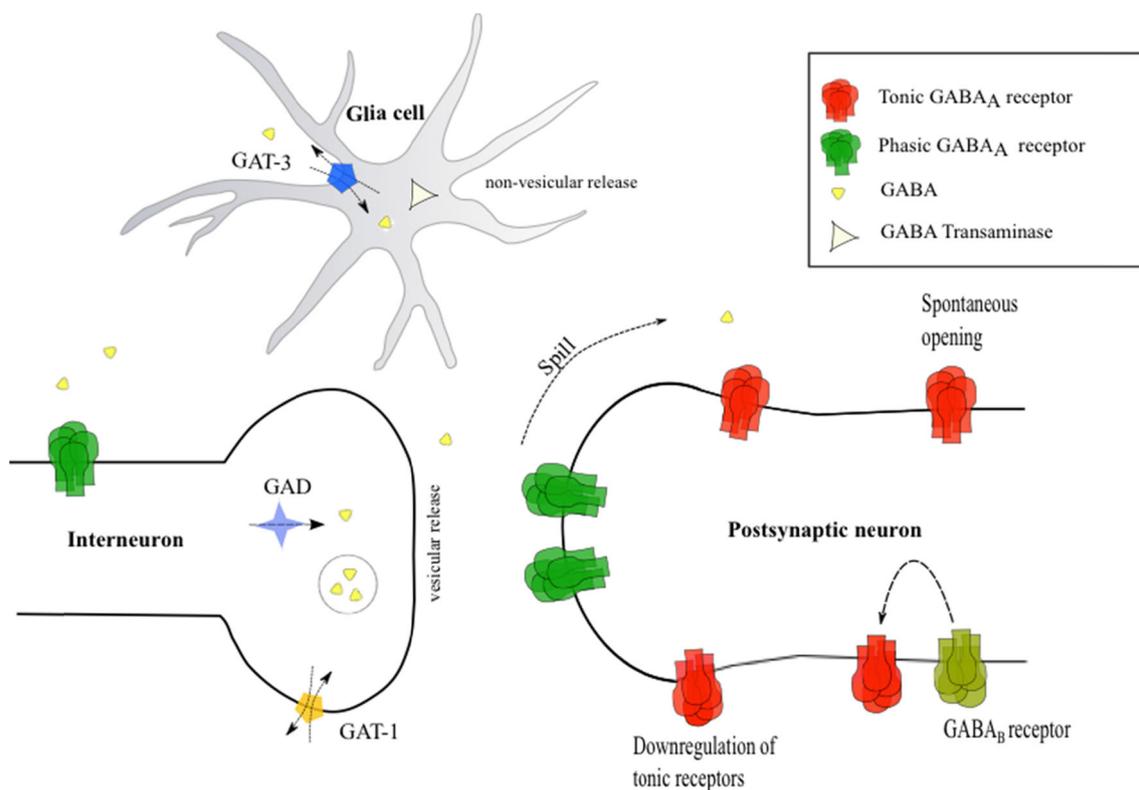


**Fig. 1** Mechanisms modulating the activity of tonic GABA$_A$Rs. Upon release into the synaptic cleft, GABA is taken up in glia cells by GABA transporter (GAT) type 3 and metabolized by GABA transaminase or taken up presynaptically by GAT-1. Tonic GABA$_A$Rs can be activated by four mechanisms: (1) spill of synaptically released GABA into the extrasynaptic space due to insufficient clearance by GATs, (2) non-vesicular GABA release by GAT reversal, (3) spontaneous opening in the absence of extracellular GABA, and (4) GABA$_B$ receptor activation increases tonic GABA$_A$R signaling via an intracellular mechanism

The mechanisms governing tonic inhibition are summarized and illustrated in Fig. 1.

## The Influence of GABA$_B$ Receptors on Tonic GABA Signaling

Metabotropic GABA$_B$Rs are expressed both, presynaptically and postsynaptically, and are known to modulate seizure activity. At the presynapse, they act as autoreceptors, i.e., activation of this receptor causes a reduced $Ca^{2+}$ entry, resulting in a decreased GABA release. At the postsynaptic site, GABA$_B$Rs can modulate tonic signaling. For instance, activation of GABA$_B$Rs by baclofen increases the tonic current of δ containing GABA$_A$Rs in thalamocortical cells, DG, and cerebellar granule cells [60]. The mechanisms by which GABA$_B$ signaling controls tonic GABA$_A$R currents are not fully understood. It is hypothesized that activation of postsynaptic GABA$_B$Rs initiates a G-protein coupled signal transduction to δ containing GABA$_A$Rs (for review, see [61]). The contribution of this pathway versus that of autoreceptors in the proconvulsive or anticonvulsive activity of GABA$_B$R-modulating drugs remains to be elucidated.

The spatial expression of GABA$_B$Rs at the postsynaptic membrane shares striking resemblance with that of extrasynaptic or perisynaptic GABA$_A$Rs. Most likely GABA$_A$Rs and GABA$_B$Rs are activated simultaneously due to spillover of GABA [34, 62, 63]. Furthermore, GABA$_B$Rs are found on astrocytes. As these cells have an essential buffering capacity, glial GABA$_B$Rs may be important in regulating the concentration of extracellular GABA [64, 65].

## Clustering and Trafficking of GABA$_A$R

The expression of GABA$_A$Rs is tightly controlled and depends on assembly, maturation, and recycling of different subunits. These processes are regulated by a complex interaction of various proteins such as GABA$_A$R-associated protein (GABARAP) and N-ethylmaleimide-sensitive factor (for a review, see Lorena Arancibia-Cárcamo 2009). Normally, GABA$_A$Rs are inserted into the cell membrane at the extrasynaptic location and diffuse via lateral trafficking into the postsynaptic density (PSD; Fig. 2). Here, they are clustered by different adhesion and scaffolding proteins, i.e., gephryin, dystrophin, and neurexin [66]. GABA$_A$Rs can also be clustered at the extrasynaptic site by molecules such as radixin [67, 68]. Receptors are able to migrate back and forth between the synaptic and extrasynaptic site [69, 70] and can rapidly cycle back after endocytosis to the extrasynaptic membrane [28]. It is unclear which mechanisms cause receptors to diffuse away from the synaptic site or facilitate their entry into the PSD.

An increase in extrasynaptic clustering influences tonic signaling. Surprisingly, not only the amount of channels but also individual channel properties change with increased clustering [71, 72]. Clustered GABA$_A$Rs have a higher $EC_{50}$, deactivate faster, and desensitize slower compared to diffuse receptors [72]. The heterogeneity of receptors and preference of certain subunits to cluster extrasynaptically complicate the understanding of this mechanism [71].

## Tonic Inhibition in Interneurons

The activity of interneurons paces the hippocampal rhythm. This rhythmogenesis is therefore partly controlled by the tonic GABA conductance in interneurons. This is demonstrated by the finding that δ subunit knockout mice show higher frequency γ oscillations [11]. However, interneurons show a bivalent response to ambient GABA. At low levels of GABA, tonic inhibition is reduced, causing an enhanced excitability of interneurons [24, 73] and consequently an increased inhibition of pyramidal cells. Moreover, this weak tonic conductance imposes regular firing of interneurons that synchronizes the CA 3 network [74]. On the other hand, high concentrations of ambient GABA inhibit interneurons [75]. As a result, they become less excitable and thus release their brake on pyramidal cells. However, under these high extracellular GABA levels, the pyramidal cells themselves also receive more tonic inhibition. This inhibition can counterbalance the loss of interneuronal inhibition. Thus, control of the extracellular GABA concentration provides both a direct and indirect mechanism to regulate pyramidal cell activity [73].

## Tonic GABA Signaling in Temporal Lobe Epilepsy

Considering the important role of tonic GABA signaling in regulating network excitability, it is likely that tonic GABA signaling is altered in the occurrence of seizures or in the development of a seizure prone network (epileptogenesis) [76, 77]. Studies on tonic GABA signaling in epilepsy will be discussed in the following paragraph and are summarized in Table 2.

Single nucleotide polymorphisms and mutations in genes coding for tonic subunits are associated with several types of epilepsy [78–80]. For instance, Dibbens et al. [78] and Feng et al. [81] showed that genetic alterations in the GABRD gene, that codes for the tonic δ subunit, cause a decrease in tonic inhibition in complex idiopathic generalized epilepsies. Eugene et al. [80] found that in human epileptic syndromes with febrile seizures, mutations in the γ subunit cause a decrease in tonic currents by reducing the surface expression of α5 containing GABA$_A$Rs.

Results on the expression levels of tonic subunits in experimental epilepsy are contradictory (for a full overview, see

**Fig. 2** Receptor trafficking to the membrane and between the extrasynaptic and synaptic site. Additionally, examples of extrasynaptic and synaptic anchoring proteins are shown
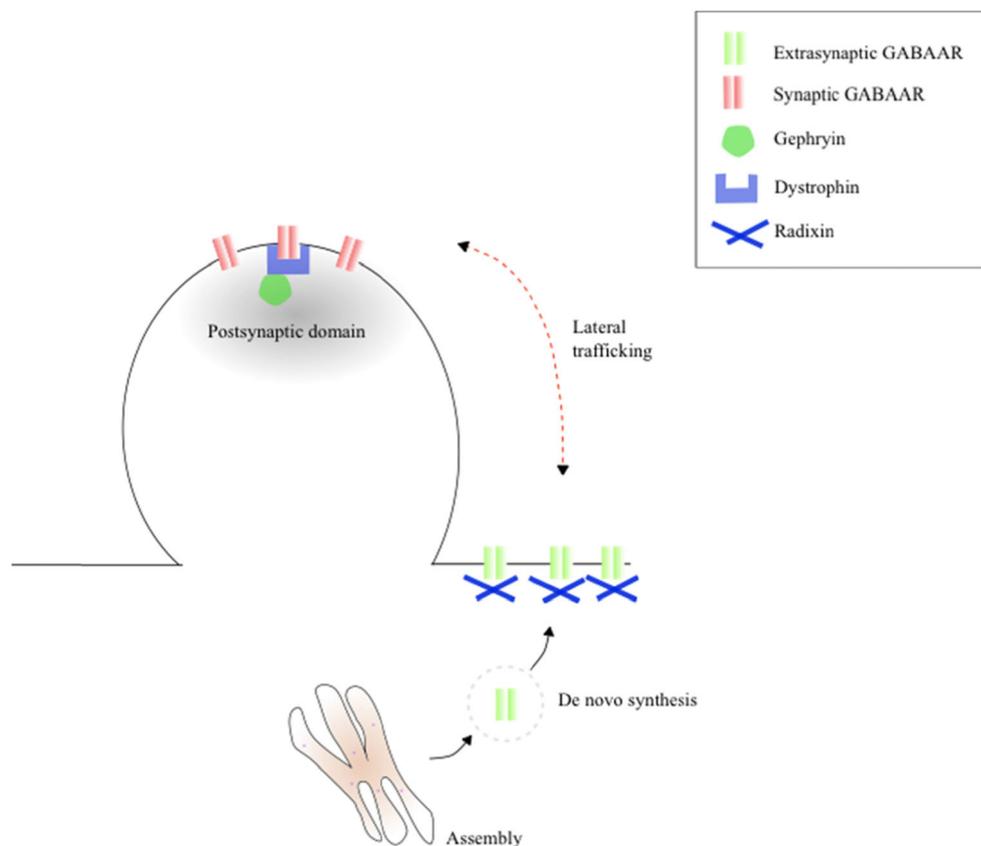


Table 2). Whereas several studies showed an increase of the α5 subunit [82–84] particularly in the DG in the kainate and pilocarpine model, others showed a decrease in CA1 in the pilocarpine model [84], in the DG in the kainate model [85] in CA1, CA2, and CA3 in the pilocarpine model [83], and in the DG, CA1, and CA3 in the hippocampal kindling model and the pilocarpine model [86–88]. The total and surface expression of the δ subunit in the DG of the hippocampus decreases after status epilepticus, both during the latent phase shortly after the induction of a status epilepticus and during the chronic phase, when animals experience spontaneous recurrent seizures [89–91]. As the expression of the δ subunit is concomitantly increased in the microsomal fraction, these results suggest that these subunits do not reach the cell membrane and are retained in the endoplasmatic reticulum. All together, is seems that there is a quantitative decrease in the amount of tonic subunit expression at the messenger RNA (mRNA) and protein level in different hippocampal regions acutely after an epileptogenic insult but also during the chronic phase.

Nonetheless, electrophysiological studies have shown that alteration in the expression of tonic subunits is not accompanied by a functional loss of tonic inhibition [89, 91–93]. Some studies even report an increase of tonic signaling in experimental epilepsy shortly after status epilepticus and during the epileptogenic phase in the pilocarpine model [88, 94]. If, as

animal studies suggest, subunits providing tonic currents are downregulated and tonic inhibition is unchanged or increased, then tonic GABA currents must be maintained by other means. What factors are possibly involved in maintaining tonic currents?

As the amplitude of tonic GABA signaling is determined by the concentration of GABA in the synaptic cleft, an increased GABA concentration could compensate for a decrease in the quantity of tonic receptor subunits. As discussed before, increased extracellular GABA concentrations can result from a reduced activity or number of GATs. Indeed, GAT-1, which is expressed presynaptically, was shown to be upregulated in the molecular layer of the hippocampus acutely after experimental status epilepticus, in the chronic phase in the kainic acid model [95] and in hippocampal specimen from patients with temporal lobe epilepsy [96]. Andre et al. [97] have shown that GAT-1 alterations depend on the time point in the epileptogenic process and the region investigated. Whereas GAT-1 was upregulated in the inner molecular layer of the hippocampus, it was downregulated in CA1 in rats with spontaneous, recurrent seizures compared to controls. Another important regulator of extracellular GABA is GAT-3, which is located on glial processes. The expression of both GAT-1 and GAT-3 are altered in hippocampi from TLE patients. As these changes vary per hippocampal subregion and GATs can reverse, causing non-vesicular GABA release, it is difficult to predict

**Table 2** Summary of the evidence concerning the role of subunit expression in temporal lobe epilepsy

| Author | Year | Model | Species | Technique | Cell type | Decrease | Increase | Additional findings |
|---|---|---|---|---|---|---|---|---|
| Bouilleret | 2000 | SE KA i.c. | Mouse | IHC | DG (GC/ML) CA1 | α5, γ2 | γ2, α5, α1 | Loss of GAT-1 in CA1 and DG, not in CA3 |
| Brooks-kayal | 1998 | SE Pilocarpine | Rat | Whole cell patch clamp, Single-cell mRNA amplification | DG | α1 (E) | α4, δ | Altered sensitivity to zolpidem and zinc Increased GAD67 expression |
| Drexel | 2013 | SE KA | Rat | In situ hybridization | DG CA1 CA3 | α5, δ; γ2 (E), δ; α5, γ2 (E) | α4, α1; α1 | |
| Fritschy | 1999 | SE Pilocarpine | Rat | IHC | DG (GC/ML) CA3 | α1; α5 | α3, α5; γ2 | Maintenance of tonic GABA currents No reduction of δ subunit expression |
| Goodkin | 2008 | SE Continuous hippocampal stimulation | Rat | Whole cell patch clamp (DG) | | | | |
| Houser | 2003 | SE Pilocarpine | Rat | IHC In situ hybridization | CA1/2 | α5 | | |
| Kamphuis | 1995 | Amygdala kindling | Rat | In situ hybridization | DG (GC) | γ2 (L) | α1/2/4 (E), γ2 (E) | |
| Lee | 2013 | SE Pilocarpine | Rat | Whole cell patch (DG) 2–3 weeks after status epilepticus | | | | Increase tonic inhibition in GC in DG |
| Loup | 2000 | Human | Human, hippocampal sclerosis | IHC | DG GC ML | | α1, γ2; α2 | |
| Naylor | 2005 | SE Pilocarpine | Rat | Whole cell patch clamp (DG) | | | | Increase in tonic GABA$_A$R mediated currents one hour after SE |
| Nishimura | 2005 | SE Hippocampal kindling Self-sustained limbic status epilepticus | Rat | In situ hybridization | DG (GC) CA1 CA3 | α5 (E/L), δ (E/L); α5 (E/L); α5 (E/L), γ2 (E) | γ2 (E) | |
| Peng | 2004 | SE Pilocarpine | Mouse | IHC | DG (ML) DG (IN) | δ | δ | |
| Rajasekeran | 2010 | SE Continuous hippocampal stimulation | Rat | Patch clamp Western blot | DG | δ | α4 | Tonic currents are maintained in DG cells post-SE by α4γ2 receptors Reduced neurosteroid (allopregnanolone, L655708) sensitivity in epileptic DGC Retention of δ subunit in ER |
| Scimemi | 2005 | SE Pilocarpine/KA | Rat | Whole cell patch clamp CA1 IHC | CA1/3 | α5 | | Maintenance of tonic GABA currents |
| Schwarzer | 1997 | SE KA | Rat | IHC | DG (ML) | α2, δ (E) | α1/2/4/5, δ, γ2 (L) | |
| Sun | 2013 | i.c. CTZ injection | Cell culture Rat | Whole cell patch clamp in cultured hippocampal | | | | Overexpression α5β3γ2 and α6β3δ resulted in enhanced |

**Table 2** (continued)

| Author | Year | Model | Species | Technique | Cell type | Decrease | Increase | Additional findings |
|---|---|---|---|---|---|---|---|---|
| | | | | neurons overexpressing α5β3γ2 and α6β3δ Field potentials in vivo | | | | tonic inhibition and reduced epileptiform activity in vitro THIP (5 μM) suppressed epileptiform burst activity and behavioral seizures |
| Tsunashima | 1997 | SE KA | Rat | In situ hybridization | DG (GC) CA1 CA3 | α5, γ2 (E) δ (L) α5, γ2 (L) α5, γ2 | | |
| Zhan | 2009 | SE Pilocarpine | Rat Mouse | Whole cell patch clamp (DG) | | | | Increase in tonic signaling Tonic currents are maintained by α4β×δ and α5β×γ |
| Zhang | 2007 | SE Pilocarpine | Mouse | Immunogold-electronmicroscopy Whole cell patch clamp | DG (GC) | δ | | Shift of γ2 toward perisynaptic location Magnitude of tonic GABA currents maintained |

*DG* hippocampal dentate gyrus, *E* early, *GC* granular cell, *i.c.* intracerebral, *IHC* immunohistochemistry, *IN* interneuron, *KA* kainic acid, *ML* molecular layer

the effect of these alterations to hippocampal physiology [63, 95].

Whether an actual functional reversal of transporters occurs in epilepsy is difficult to establish due to the technical restrictions of measuring intracellular GABA concentrations. GAT reversal is eventually favored by an increased rate of GABA synthesis by GAD [95] that in its turn can be caused by an increase in metabolic rate of neurons as seen in epilepsy. Another possibility of increasing extracellular GABA is by increased activity of glutamic acid decarboxylase (GAD), the enzyme responsible for GABA synthesis. Esclapez and Houser [98] have shown that GAD 65 and 67 are increased in animals with spontaneous, recurrent epileptic activity at the mRNA and protein level. Acutely after status epilepticus, GAD 65 and 67 expression decreased in the hippocampal hilus, whereas GAD 67 expression in the DG is increased [99]. In the chronic phase, GAD accumulated in interneurons of the DG [82, 100]. Interestingly, GAD 65 null mice show spontaneous seizures [101].

Additionally, different channels (e.g., two-pore domain potassium channels) and receptors or GABA$_A$Rs with a different subunit composition could take over tonic function [74, 102]. Indeed, pharmacological experiments demonstrated that increased tonic GABA currents are not mediated by α5- but δ-containing GABA$_A$Rs in epilepsy [88]. In several studies, it has been shown that the down-regulation of δ subunits in the DG is accompanied with an increase in the expression of receptors containing the α4 and γ2 subunits [86, 89, 90, 103]. As mentioned before, these receptors have a lower affinity for GABA, which implies that tonic GABA currents can only be maintained if there is an increase in extracellular GABA. Possibly, receptors that are typically expressed synaptically under physiological conditions are increasing shifted toward the extrasynaptic site in epilepsy. Evidence for this process comes from the shift of the γ2 subunit that is increasingly expressed at the extrasynaptic site in a murine model of epilepsy [104]. Additional electrophysiological studies are necessary to fully understand the functional consequences of quantitative changes in GABA$_A$Rs.

## Toward Antiepileptic Drugs that Target Tonic GABA Signaling

Considering that tonic currents are functionally preserved in epilepsy, they constitute a potential treatment target [5, 15]. In order to increase tonic currents, there are two strategies: increasing the extracellular concentration of GABA or agonizing GABA$_A$Rs by ligands binding to tonic subunits. In Fig. 1, the mechanisms modulating tonic signaling are displayed. These mechanisms

will be discussed in the light of increasing tonic inhibition in the next paragraph.

## Increasing the Extracellular GABA Concentration

Extracellular GABA concentrations are controlled by synthesis, breakdown, and clearance from the synaptic cleft. The synthesis of GABA in the presynaptic terminal is determined by the activity of GAD 65, and the activity of single GAD is influenced by processes such as phosphorylation and the presence of co-factors such as pyridoxal 5′-phosphate. GAD concentrations can be increased by enhancing its transcription by stimulating promoter activity or by influencing epigenetic mechanisms that lead to a higher transcription rate [89, 105]. Alternatively, GAD can be potentiated by viral vector-mediated targeted delivery of therapeutic GAD. This strategy has already proven to be successful in experimental and clinical studies for Parkinson's disease [106–108]. An advantage of viral vector-mediated targeted delivery therapy is its spatial specificity that potentially eliminates site effects caused by unspecific targeting.

Alternatively, a higher release of GABA could be achieved by increasing the concentration of GABA in individual presynaptic vesicles. GABA is packed into vesicles by vesicular neurotransmitter transporters (VGAT), and their activity depends on electrochemical components. Boosting vesicular GABA transport might be achieved by pharmacologically increasing the activity of VGAT or by increasing its gene expression.

A second mechanism to increase extracellular GABA concentrations is inhibiting breakdown of GABA that is mediated by GABA transaminase (GABA-T). The second-line AED vigabatrin works via irreversibly inhibiting GABA-T [109, 110], increasing the concentration GABA in the synaptic cleft [111]. Furthermore, there is evidence that vigabatrin increases GABA release and constrains glial GABA uptake [112, 113]. Other more potent and novel inhibitors of GABA-T are ethanolamine-O-sulfate (EOS), L-cycloserine, and phenylethylidenehydrazine. Interestingly, the latter is used in various psychiatric disorders and increase extracellular GABA concentrations in vivo and decrease epileptiform activity in the rat ex vivo [114]. However, its anticonvulsant properties in vivo varied depending on the seizure model used. Several other GABA-T inhibitors such as gabaculine, gamma-acetylenic GABA, and gamma-vinyl GABA do not appear suitable for treatment of convulsive disorders in humans due to their severe and sometimes lethal side effects [115].

The last mechanism to increase extracellular GABA concentrations is by decreasing GABA clearance from the synaptic cleft by influencing GAT. Two drugs acting at GATs are SNAP-5114 and tiagabine that inhibit GAT-2 and -3, and GAT-1, respectively [116]. Indeed, they increase the concentration of extracellular GABA in vivo [117]. Tiagabine seems to be efficient as an add-on treatment in partial and secondarily generalized seizures, reducing seizure frequency [118]. However, clinical data show disappointing results with patients suffering from paradoxical proconvulsive effects [119]. So far, no pharmaceutical interventions exits that acts by reversing GATs.

## Extrasynaptic GABA$_A$R Agonists

In addition to enhancing extracellular GABA, enhancement of tonic GABA$_A$Rs can also be achieved by using specific agonists. Ganaxolone is a neurosteroid and a tonic GABA$_A$R agonist, binding to and influencing the δ subunit. At low concentrations, it potentiates GABA$_A$Rs (positive allosteric modulator), while at higher doses, it acts by directly binding to the δ subunit. Several studies using ganaxolone show promising results, both in rodent seizure models [120–122] as well as in clinical studies [123]. In patients with infantile spasms, seizure frequency was decreased by at least 50 % in one third of the patients [124]. The frequency of occurrence and type of side effects were comparable to classic antiepileptic drugs. Currently, ganoxalone is investigated as an adjunctive treatment in patients with drug-resistant, partial onset seizures (trial identifier NCT01963208).

Also, the anesthetic 4,5,6,7-tetrahydroisoxazolo(5,4-c)pyridin-3-ol (THIP, gaboxadol) agonizes the δ subunit. THIP can reduce spikes in vitro and in vivo [125, 126]. In a clinical study with a small sample size, a trend toward seizure reduction was demonstrated [127]. However, there is conflicting evidence showing no effect on epileptiform activity [128, 129]. Other anesthetics, which selectively target the δ subunit are alphaxalone and propofol. Due to its anesthetic effects, these drugs are not suitable for the treatment of epilepsy.

A relatively new compound binding selectively to the δ subunit is the GABA agonist DS-1 and an enhancer DS-2. DS-2 has been shown to increase tonic currents in the thalamic neurons in vitro [130].

## Challenges of Enhancing Tonic Signaling

The development of AEDs aimed at tonic receptors is not straightforward due to several reasons. First of all, the long-term use of tonic GABA$_A$R agonists, in particular neurosteroids, can lead to a downregulation of α4 and δ subunits. The elimination of the binding site by downregulation ceases the potential long-term benefits of this strategy. Furthermore, AEDs can have side effects. Side effects depend on the concentration of the compound and the distribution in the central nervous system. However, one advantage of extrasynaptic GABA$_A$Rs is their cell type and brain region-specific subunit composition. The advantage of this heterogeneity of receptors is that all subunits differ in their affinity for agonists and antagonists. The aspecificity of drugs can

therefore be partially overcome by using different dosages of AEDs. For instance, δ subunit containing receptors have the highest affinity for GABA.

Furthermore, compensatory alterations in other cells or brain regions could be a cause of ineffectiveness of AEDs. For instance, increasing GABA concentrations through inhibition of GAT-3 is not effective in the hippocampus due to the compensatory action of GAT-1. In the thalamus, however, GAT-3 inhibition increases tonic signaling. Related to these region-specific effects is the fact that certain types of epilepsy appear to be more suitable targets for tonic GABA modulation. For instance, in the absence of seizures, tonic inhibition in the thalamus is upregulated rather than downregulated [76]. Enhancing tonic signaling triggers slow wave discharges—a hallmark of this type of epilepsy—and consequently aggravates seizures [131]. In this light, it becomes plausible that an increase in tonic inhibition particularly in the thalamus causes absence-like side effects.

It is important to realize that an increase in extracellular GABA has consequences for synaptic receptors and receptors on which GABA acts as a (partial) ligand (i.e., GABA$_B$Rs). In this regard, it might be sometimes difficult to disentangle whether the specific increase in tonic currents or a gross enhancement in the inhibitory tone causes antiepileptiform effects.

## Future Directions for the Development of Tonic AEDs

To overcome the challenges described above, it is necessary to reveal the complex network effects of tonic signaling and develop more specific and targeted drugs. By targeting specific subunits, one could make use of the particular pharmacokinetic properties such as affinity, channel opening time, refractory period, etc., which would allow fine-tuning of neuronal activity in the presence of varying concentrations of antagonist or agonists [23, 24, 88, 132].

Potential new specific strategies are evolving. An example of a cell-type specific therapy is optogenetics [133]. With optogenetics, certain genetically manipulated neurons are inhibited or excited by light. If one succeeds in selectively inhibiting the principal neuron only in the epileptogenic zone or increasing the activity of interneurons, this would potentially create new treatment opportunities. In the case of epilepsy, it could be beneficial to increase the release of GABA and therefore increase ambient GABA concentrations, which, in turn, can activate extrasynaptic receptors. Considering the important buffering capacity of astroglia cells, those might also form an attractive target with regard to regulating the concentration of extracellular GABA.

Additionally, by using cell-specific approaches, side effects could be limited. Also, designer receptors exclusively activated by designer drugs (DREADDs) might evolve as future treatment strategy [134]. With DREADDs, receptors are administered to the central nervous system via a viral vector into specific cell types. A specific oral drug activates these receptors. In fact, in epilepsy, there are two options with regard to DREADDs [135]. On one hand, inserting the receptor into the presynaptic gabaergic terminal and activating it by the oral drug could increase presynaptic GABA release and therefore augment not only tonic but also phasic inhibition. On the other hand, the precise activation of certain interneuron populations in the epileptogenic zone might contribute to a higher excitation threshold of the principal neuron, which, in turn, might be beneficial at the network level. The advantage of this procedure is the specificity of the treatment and fact that it is less invasive compared to optogenetic techniques.

Another strategy might be to interfere with receptor trafficking. A potential approach would be to enhance tonic signaling by increasing receptor expression and anchoring. This could be achieved by stimulating de novo synthesis or by promoting the migration of receptors from the synaptic to the extrasynaptic site. A potential consequence might be the loss of synaptic receptors, which, in turn, leads to the loss of phasic inhibition, which is detrimental to epilepsy. Next to increasing de novo synthesis, shuttling of ready-made receptors to the cell surface could be enhanced.

Lastly, it might be desirable to improve anchoring at the extrasynaptic side. In this regard, anchoring proteins such as radixin might play a crucial role. Whereas disrupting certain protein-protein interactions becomes more feasible by using virus-derived proteins [136, 137], enhancing these interactions is more difficult.

## Conclusion

Tonic inhibition is maintained in epilepsy and therefore serves as an attractive substrate for interventions. Drugs aimed at tonic GABA signaling, such as ganoxalone, show promising anticonvulsive results in rodent studies, but they trigger side effects as well. Future detailed knowledge about receptor trafficking and particular changes in specific brain regions will contribute to more rational drug design leading to more potent drugs with fewer side effects.

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