Minireviews

Genetic and Molecular Regulation of Extrasynaptic GABA-A Receptors in the Brain: Therapeutic Insights for Epilepsy

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Received August 17, 2017; accepted November 13, 2017

ABSTRACT

GABA-A receptors play a pivotal role in many brain diseases. Epilepsy is caused by acquired conditions and genetic defects in GABA receptor channels regulating neuronal excitability in the brain. The latter is referred to as GABA channeopathies. In the last two decades, major advances have been made in the genetics of epilepsy. The presence of specific GABAergic genetic abnormalities leading to some of the classic epileptic syndromes has been identified. Advances in molecular cloning and recombinant systems have helped characterize mutations in GABA-A receptor subunit genes in clinical neurology. GABA-A receptors are the prime targets for neurosteroids (NSs). However, GABA-A receptors are not static but undergo rapid changes in their number or composition in response to the neuroendocrine milieu. This review describes the recent advances in the genetic and neuroendocrine control of extrasynaptic and synaptic GABA-A receptors in epilepsy and its impact on neurologic conditions. It highlights the current knowledge of GABA genetics in epilepsy, with an emphasis on the neuroendocrine regulation of extrasynaptic GABA-A receptors in network excitability and seizure susceptibility. Recent advances in molecular regulation of extrasynaptic GABA-A receptor-mediated tonic inhibition are providing unique new therapeutic approaches for epilepsy, status epilepticus, and certain brain disorders. The discovery of an extrasynaptic molecular mechanism represents a milestone for developing novel therapies such as NS replacement therapy for catamnial epilepsy.

Introduction

Alterations in the structure and function of neurotransmitter receptors play critical roles in the pathophysiology of many brain diseases. Epilepsy is one of the most widespread and debilitating neurologic disorders, affecting approximately 3.4 million people in the United States and 65 million people worldwide. This disorder is a chronic condition characterized by two or more unprovoked seizures occurring due to excessive or hypersynchronous electrical discharge of neurons in the brain (Hauser 1994; Thurman et al., 2011; Hesdorffer et al., 2013). Many subregions and a wide variety of neurotransmitters are involved in the pathology of epileptic seizures. The GABA-A receptor, a subtype of receptor activated by the inhibitory neurotransmitter GABA, is a prime target for many seizure medications. Although antiepileptic drugs allow for symptomatic control of seizures, epilepsy remains incurable, partially due to our poor understanding of the molecular and electrophysiological basis of epilepsy development. Advances in our understanding of the pathology of epilepsy are crucial for discovering effective treatments for epilepsy and related brain disorders.

Current knowledge indicates that approximately 60% of epilepsy is idiopathic and 40% stems from developmental or

ABBREVIATIONS: AP, allopregnanolone (3α-hydroxy-5α-pregnan-20-one); BLA, basolateral amygdala; bp, base pair; CAE, childhood absence epilepsy; CaMKII, Ca2+/calmodulin-dependent protein kinase II; CNS, central nervous system; DGGC, dentate gyrus granule cell; DHEAS, dehydroepiandrosterone sulfate; DKO, GABA-A receptor β subunit knockout; DS, Dravet syndrome; ER, endoplasmic reticulum; FS, febrile seizure; GABRA, gene encoding the GABA-A receptor α subunit; GABRB, gene encoding the GABA-A receptor β subunit; GABRD, gene encoding the GABA-A receptor δ subunit; GABRG, gene encoding the GABA type A receptor γ subunit; GEFS+, generalized epilepsy with febrile seizures plus; GX, ganaxolone (3α-hydroxy-3β-methyl-5α-pregnan-20-one); HEK, human embryonic kidney; JME, juvenile myoclonic epilepsy; mIPSC, miniature inhibitory postsynaptic current; NS, neurosteroid; OP, organophosphate; PKA, protein kinase A; PKC, protein kinase C; SE, status epilepticus; THDOC, alloetotohydrodeoxyxictocortosterone (3α,21-dihydroxy-5α-pregnan-20-one); THIP, gaboxadol (4,5,6,7-tetrahydroisoxazolo-[5,4-c]pyridin-3-ol); TM, transmembrane domain; WT, wild-type.
acquired conditions such as a stroke, traumatic brain injury, infections, tumor, drug withdrawal, neurotoxicity, or prolonged seizures and genetic defects (Eslami et al., 2016; Pitkänen et al., 2016; Vezzani et al., 2016). The underlying mechanisms that render a normal brain to progressively develop into a brain with recurring seizures are still elusive. Along with age- and sex-related factors, other physiologic abnormalities in neurotransmitter release, functions of ion channels, synaptic connectivity, neural circuitries, or the interaction of these factors facilitate seizure development. The term “epileptogenesis” is used to describe the complicated process of the development of acquired epilepsy. Epileptogenesis denotes a plastic progression in which the balance of neuronal excitation/inhibition, neuronal interconnections, and neuronal circuits undergo gradual changes during or after a series of insults and, consequently, transform a normal brain into one that is hyperexcitable, suffers neuronal loss and damage, and, as a result, has recurrent spontaneous seizures.

In this article, we describe briefly the emerging concepts on GABA genetics in epilepsy, with a special emphasis on the functional role of extrasynaptic GABA-A receptors in the regulation of network excitability and susceptibility to brain disorders. We also highlight the potential therapeutic implications of modulating extrasynaptic GABA-A receptor–mediated tonic inhibition in pharmacotherapy of epilepsy, status epilepticus (SE), and other brain disorders.

**Molecular Pharmacology of Neuronal GABA-A Receptors**

**GABA-A Receptor Subtypes.** GABA is the most abundant inhibitory neurotransmitter in the brain. It is a highly hydrophilic molecule and hence cannot cross the blood-brain barrier. GABA is synthesized in neurons and stored in synaptic vesicles. Upon neuronal activation, GABA is released from the vesicles into the synapse, where it can act on postsynaptic receptors, or diffuse into the extracellular space. GABA binds with three receptors: GABA-A, GABA-B, and GABA-C. GABA-A receptor plays a pivotal role in regulating neuronal excitability and in the pathology of epilepsy (Baulac et al., 2001). GABA exerts fast inhibitory actions by activating postsynaptic GABA-A receptors in the brain, causing the influx of negatively charged chloride ions and hyperpolarization of neurons which serve to reduce neuronal excitability and firing. GABA-A receptors are pentamers consisting of five subunits. Each subunit has one long extracellular N terminus that interacts with a variety of drugs, including benzodiazepines, barbiturates, and neurosteroids (NSs); four transmembrane domains (TMs) (TM1–TM4); and one short intracellular loop that links TM1 and TM2, one short extracellular loop that links TM2 and TM3, one long intracellular loop that links TM3 and TM4 and can be modulated by phosphorylation, and one small extracellular C terminus. The TM2 of each subunit forms a selective channel pore that is permeable for the chloride ion passage (Fig. 1). GABA-A receptor isofrom distribution plays key roles in regulation of sedation, hypnosis, anxiolysis, anesthesia, and seizure protection (Table 1).

GABA-A receptors are made from a repertoire of 19 known subunits, as follows: α1–6, β1–3, γ1–3, δ, ε, θ, π, and ρ1–3. The most general stoichiometry of GABA-A receptors contains two α subunits, two β subunits, and one γ subunit, or one δ subunit. GABA-A receptor subunits have discrete distributions among different brain regions. Approximately 90% of GABA-A receptors are γ-containing, but the δ subunit can substitute for γ. The δ subunit has more confined expression in parts of the brain such as the hippocampus, cerebellum, and thalamus. GABA binding sites are located at the junction between subunits α and β, and benzodiazepines bind at the interface between subunits α and γ. The genomic location of 19 GABA-A receptor subunits has been identified (Russek, 1999). The genes encoding subunits α2, α4, β1, and γ1 cluster on chromosome 4p12; subunit α1, α6, β2, γ2, and π genes are mapped on chromosome 5q; subunit α3, β3, and γ3 genes cluster on chromosome 15q12; subunit α3, θ, and ε are located on chromosome Xq28; ρ1 and ρ2 cluster on chromosome 6q15; and the β3 and δ subunits are found on chromosome 3q11.2 and chromosome 1p36.3, respectively (Fig. 2).

**Synaptic Versus Extrasynaptic GABA-A Receptors.** GABA-A receptors are divided into two categories according to their localization: synaptic and extrasynaptic receptors. Each type of receptor possesses distinct characteristics in its affinity and efficacy to GABA, desensitization rate, and response to benzodiazepines and NSs (Bianchi and Macdonald, 2002, 2003; Brown et al., 2002; Wohlfarth et al., 2002; Mortensen et al., 2012). Extrasynaptic GABA-A receptors are composed of mainly δ-containing receptors, which have high GABA affinity but low efficacy, low desensitization rate, and low sensitivity to benzodiazepines, and are highly potentiated by NSs compared with synaptic GABA-A receptors (Table 2). Activation of synaptic γ-containing GABA-A receptors and extrasynaptic δ-containing GABA-A receptors produce phasic and tonic current inhibition, respectively. Rapid and transient phasic current inhibition is generated by presynaptic GABA release and the binding of synaptic γ-containing GABA-A receptors, whereas tonic current inhibition is produced by persistent activation of perisynaptic or extrasynaptic δ-containing GABA-A receptors by ambient GABA. Extrasynaptic receptors are only found in specific brain areas such as the hippocampus, amygdala, neocortex, thalamus, hypothalamus, and cerebellum (Stell et al., 2003; Jia et al., 2005; Drasbek and Jensen, 2006; Olmos-Serrano et al., 2010; Mortensen et al., 2012; Carver et al., 2014). Extrasynaptic δ-containing GABA-A receptors are tailored to regulate neuronal excitability by controlling the basal tone through shunting and tonic inhibition in neurons (Coulter and Carlson, 2007; Carver and Reddy 2013). They are mostly insensitive to allosteric modulation by benzodiazepines such as midazolam (Reddy et al., 2015; Carver and Reddy, 2016).

NSs are powerful modulators of GABA-A receptors and can rapidly alter neuronal excitability (Reddy and Estes, 2016). NSs act at both synaptic and extrasynaptic GABA-A receptors, but they are more efficacious on extrasynaptic δGABA-A receptors that mediate tonic inhibition. At low concentrations (submicromolar level), NSs allosterically potentiate GABA-A receptor currents, whereas at high concentrations (micromolar level), NSs can directly activate GABA-A receptors by binding directly at the orthosteric site (Reddy and Rogawski, 2002; Hosie et al., 2007; Reddy and Jian, 2010; Carver and Reddy, 2016). One recent study (Joshi et al., 2017) demonstrated a downregulation of the δ-containing GABA-A receptors prior to the onset of epilepsy, and a reduction in NS-induced modulation of tonic inhibition in the SE model of epilepsy, highlighting the role of δGABA-A receptors in this disease.
Different subtype combinations respond differently to receptor modulators and contribute distinct functions in each brain area. GABA-A receptors have a high molecular heterogeneity because of the abundance of available subunits and the ways in which those subunits can be assembled to form the heteropentameric receptors. The composition and distribution of the GABA-A receptor isoforms are listed in Table 3. Receptors located in the synaptic sites are primarily made of α, β, and γ2 subunits. α1, α2, α3, and α5-containing receptors are generally sensitive to benzodiazepines. γ Subunit–containing receptors constitute the majority of GABA-A receptors. Immunohistochemistry and in situ hybridization data showed that α1β2γ2 is the most abundant and widespread subtype, accounting for ~43%–60% of all GABA-A receptors in the adult brain (McKernan and Whiting, 1996; Loup et al., 2000; Möhler et al., 2002). It mostly appears in the synaptic sites. α2β3γ2 and α3β3γ2 receptors, located mostly in the synaptic sites, are also highly prevalent. They represent ~15%–20% and ~10%–15% of all GABA-A receptors, respectively. α4γγ and α4γβ, located in the synaptic and extrasynaptic locations, respectively, represent approximately 5%, whereas synaptic α5β1/3γ2 and α6β2γ2 account for less than 5% (McKernan and Whiting, 1996; Pirker et al., 2000; Möhler et al., 2002). Other subtypes have more specific and confined distribution patterns (Fig. 3).

Receptors located in the extrasynaptic locations usually contain the δ subunit rather than the γ subunit in association with β2 or β3 and a certain isoform of the α subunit. The δ subunit with its highly specific regional and subcellular distribution is tailored to generate tonic inhibition. It is abundant in cerebellar and dentate gyrus granule cells (DGGCs), some cortical neurons, and thalamic relay cells (Wisden et al., 1992; Fritschy and Mohler, 1995; Sperk et al., 1997; Pirker et al., 2000; Peng et al., 2002).

In the hippocampus, several isoforms are expressed in distinct regions and cell types. CA1 pyramidal neurons mainly contain α5β3γ2S receptors, which are located at both synaptic and extrasynaptic sites. However, DGGCs, which have the second highest density of δ subunits, express α3β3γ2S and α4β2β3δ receptors in the synaptic and extrasynaptic site.

### Distribution of GABA-A Receptor Subtypes

<table>
<thead>
<tr>
<th>Subunit</th>
<th>α1</th>
<th>α2</th>
<th>α3</th>
<th>α4</th>
<th>β2</th>
<th>β3</th>
<th>γ2</th>
<th>δ</th>
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<tbody>
<tr>
<td>Anxiety</td>
<td>+</td>
<td>-</td>
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<tr>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
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<td></td>
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<tr>
<td>Effects of benzodiazepines</td>
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<td>-</td>
<td>-</td>
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<td>-</td>
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<td></td>
</tr>
<tr>
<td>Motor impairment</td>
<td>-</td>
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<td>-</td>
<td>-</td>
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<td></td>
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<tr>
<td>Anxiolysis</td>
<td>-</td>
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<tr>
<td>Myorelaxation</td>
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<tr>
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<tr>
<td>Effects of neurosteroids</td>
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<tr>
<td>Motor impairment</td>
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<tr>
<td>Hypnosis</td>
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<tr>
<td>Anticonvulsant</td>
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<tr>
<td>Sedation</td>
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<td>-</td>
<td>+</td>
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### TABLE 1

The pharmacological roles of select GABA-A receptor subunits

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**Fig. 1.** Schematic representation of typical GABA-A receptor (GABA-AR) structure and subunit composition. (A) GABA-ARs are heteropentamers forming a channel that is permeable to chloride ion passage. (B) A top view of the pentamer. GABA-ARs are made from a repertoire of 19 known subunits: α1–6, β1–3, γ1–3, δ, ε, θ, π, and ρ1–3. The most general stoichiometry of GABA-ARs contains two αs, two βs, and one γ; and the δ subunit can be substituted by δε, δθ, δπ, or δρ. Each subunit has four TMbs (TM1–TM4). TMbs form a selective channel pore. GABA exerts fast inhibitory actions by activating postsynaptic GABA-ARs in the brain, causing the influx of negatively charged chloride ions and hyperpolarization of neurons, which serve to reduce neuronal excitability and firing. The GABA binding sites are located at the junction between subunit α and β, whereas benzodiazepines (BZs) bind at the interface between subunits α and γ. Barbiturates binding sites are distinct from the BZ binding site. The NSs have two putative binding sites including allosteric and direct binding sites. The allosteric binding site is located at the α subunit TMs, whereas the direct binding site is within the TMbs of the α and β subunits. (C) GABA-ARs belong to the Cys-loop family of ligand-gated ion channels, which also contains nicotinic acetylcholine, glycine, and serotonin 5-HT3 receptors. Each subunit has one long extracellular N terminus that interacts with a variety of drugs including BZs, barbiturates, and NSs; four TMbs (TM1–TM4); and one short intracellular loop that links TM1 and TM2, one short extracellular loop that links TM2 and TM3, one long intracellular loop that links TM3 and TM4 and can be modulated by phosphorylation, and one small extracellular C terminus.
The delta subunit is thought to be predominantly colocalized and assembled with the alpha 4 and/or alpha 6 subunit. Alpha 4beta2/3delta or alpha 6beta2/3delta combinations exhibit the highest GABA affinity with a GABA EC50 in the nanomolar range. Alpha 1beta2delta receptors are primarily present in hippocampal interneurons and show lower GABA potency compared with the alpha 4beta2/3delta combination. Overall, alpha 1, alpha 4, gamma 2, and delta subunits are found to be expressed in the extrasynaptic sites of hippocampus (Sun et al., 2004; Glykys and Mody, 2007; Zheleznova et al., 2009). Notably, alpha 6-containing receptors are almost exclusively expressed in the cerebellar granule cells and, in combination with the delta subunit, exhibit the highest GABA potency. In the thalamus, alpha 4beta3gamma2delta receptors are expressed at synaptic sites in the thalamic relay cells, whereas, alpha 4beta3 and alpha 4beta3delta receptors are expressed at extrasynaptic sites. In addition, alpha 3delta receptors are also found in the thalamus.

In the amygdala, the alpha 2 subunit, primarily found in principal neurons, is responsible for the allosteric action of the benzodiazepines (Marowsky et al., 2004). The principal neurons in the basolateral amygdala (BLA) contain alpha 1beta2delta subunits, which contribute to the fast synaptic inhibition. Alpha 1beta2delta subunits are thought to be assembled in the principal neurons of the BLA in the synaptic site and generate phasic inhibition. In particular, mainly of the BLA in the synaptic site and generate phasic inhibition. The benzodiazepine-sensitive subunits, which contribute to the fast synaptic inhibition in principal neurons in the basolateral amygdala (BLA) also contain the benzodiazepines (Marowsky et al., 2004). The principal subunit, is responsible for the allosteric action of the benzodiazepines (Marowsky et al., 2004). The principal neurons, is responsible for the allosteric action of the benzodiazepines (Marowsky et al., 2004).

In in vitro studies, HEK293 cells expressing this mutant GABA-A receptor (alpha1A322D) exhibited reduced GABA-evoked currents, GABA sensitivity, and alpha 3subunit protein expression, along with altered current kinetics and accelerated receptor endocytosis and endoplasmic reticulum (ER)-associated degradation via the ubiquitin-proteasome system (Cossette et al., 2002; Gallagher et al., 2004, 2007; Krampfl et al., 2005; Bradley et al., 2008; Ding et al., 2010). One study (Ding et al., 2010) indicated that this mutant GABA-A receptor (alpha1A322D) causes a dominant negative effect on the composition and surface expression of wild-type (WT) GABA-A receptors. Therefore, the A322D mutation in human GABRA1 results in a loss of function of alpha 1beta2/3gamma2delta are the predominant synaptic receptor subtypes that produce phasic inhibition, whereas receptors containing alpha 4, alpha 5, alpha 6, or delta subunits (alpha 4beta3, alpha 6delta, and alpha 5gamma2delta) are the primary extrasynaptic receptor subtypes that generate tonic inhibition.

**Genetic Regulation of GABA-A Receptors**

**GABA Epilepsy Genetics.** GABA-A receptors play a pivotal role in regulating neuronal inhibition in the CNS. Dysregulation of neuronal activity and changes in the composition and function of GABA-A receptors contribute to the development of epilepsy. Genetic epilepsies are genetically driven recurrent seizures caused by mutations in genes governing excitation and inhibition. Mutations in GABA-A receptor subunit genes have been involved in the pathophysiology of several idiopathic generalized epilepsies. Specifically, mutations in the gene encoding the GABA-A receptor alpha 1 subunit (GABRA1) and the gene encoding the GABA-A receptor beta 3 subunit (GABRB3) are mainly associated with childhood absence epilepsy (CAE) and juvenile myoclonic epilepsy (JME), whereas mutations in the gene encoding the GABA-A receptor gamma 2 subunit (GABRG2) and the gene encoding the GABA-A receptor delta subunit (GABRD) are associated with febrile seizures (FSEs), generalized epilepsy with FS plus (GEFS plus), and Dravet syndrome (DS) (Fig. 4; Table 4).

**Mutations of alpha 1 Subunit.** A heterozygous missense mutation (A322D) in the TM3 of GABRA1 was identified in an autosomal dominant form of JME in a French Canadian family. This mutation arises by the replacement of the alanine amino acid residue by a larger negatively charged aspartate in the helix of TM3 of the alpha 1 subunit. In vitro studies, HEK293 cells expressing this mutant GABA-A receptor (alpha1A322D) exhibited reduced GABA-evoked currents, GABA sensitivity, and alpha 1 subunit protein expression, along with altered current kinetics and accelerated receptor endocytosis and endoplasmic reticulum (ER)-associated degradation via the ubiquitin-proteasome system (Cossette et al., 2002; Gallagher et al., 2004, 2007; Krampfl et al., 2005; Bradley et al., 2008; Ding et al., 2010). One study (Ding et al., 2010) indicated that this mutant GABA-A receptor (alpha1A322D) causes a dominant negative effect on the composition and surface expression of wild-type (WT) GABA-A receptors. Therefore, the A322D mutation in human GABRA1 results in a loss of function of alpha 1beta2/3gamma2delta are the predominant synaptic receptor subtypes that produce phasic inhibition, whereas receptors containing alpha 4, alpha 5, alpha 6, or delta subunits (alpha 4beta3, alpha 6delta, and alpha 5gamma2delta) are the primary extrasynaptic receptor subtypes that generate tonic inhibition.
### TABLE 3

<table>
<thead>
<tr>
<th>Subunit Isoforms</th>
<th>Cellular Distribution</th>
<th>Main Brain Locations/Cell Types</th>
<th>GABA Potency (EC50)</th>
<th>Pharmacological Characterization</th>
<th>References</th>
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<tr>
<td></td>
<td><strong>S</strong></td>
<td></td>
<td>µM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a1β2γ2S</td>
<td>S</td>
<td>Ubiquitously express throughout the brain. Most abundant. Constitute ~45%–60% of all GABA-A receptors</td>
<td>6.6</td>
<td>Benzodiazepines, zolpidem, and flumazenil sensitive</td>
<td>Sieghart (1995), McKernan and Whiting (1996), Pirker et al. (2000), Möhler et al. (2002)</td>
</tr>
<tr>
<td>a1β3γ2S</td>
<td>Widespread</td>
<td></td>
<td>2.1</td>
<td>Benzodiazepines and zolpidem sensitive</td>
<td>Sieghart (1995), Möhler et al. (2002)</td>
</tr>
<tr>
<td>a3β3γ2S</td>
<td>Account for 10%–15% of all GABA-A receptors. Hippocampal DGCCs, hypothalamic nuclei, thalamic reticular nucleus, cerebral cortex</td>
<td>12.5</td>
<td>Benzodiazepines, zolpidem, and flumazenil sensitive</td>
<td>Sieghart (1995), Möhler et al. (2002), Mortensen et al. (2012)</td>
<td></td>
</tr>
<tr>
<td>a4β3γ2S</td>
<td>Hippocampus, thalamic relay cells</td>
<td>2.1</td>
<td>Furosemide and Ro15-4513 sensitive</td>
<td>Brown et al. (2002), Möhler et al. (2002), Mortensen et al. (2012)</td>
<td></td>
</tr>
<tr>
<td>a6β3γ2S</td>
<td>Account for ~2% of all GABA-A receptors. Cerebellum granule cells</td>
<td>0.17</td>
<td>Furosemide and Ro15-4513 sensitive</td>
<td>Brown et al. (2002), Möhler et al. (2002), Mortensen et al. (2012)</td>
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</tr>
<tr>
<td><strong>E</strong></td>
<td></td>
<td></td>
<td>µM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a1β2δE</td>
<td>E</td>
<td>Hippocampal interneurons</td>
<td>3.7</td>
<td></td>
<td>Sun et al. (2004), Gilykys et al. (2007)</td>
</tr>
<tr>
<td>a3/5βδE</td>
<td>E</td>
<td>BLA principle neurons</td>
<td>4.5</td>
<td></td>
<td>Marowsky et al. (2004), Mortensen et al. (2012)</td>
</tr>
<tr>
<td>a3βδE</td>
<td>E</td>
<td>Thalamus, hypothalamic, locus coeruleus</td>
<td>0.97</td>
<td></td>
<td>Brickley et al. (1999), Mortensen et al. (2012)</td>
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<tr>
<td>a4β2δ3δE</td>
<td>E</td>
<td>Hippocampal DGCCs, thalamic relay cells, neostriatum</td>
<td>0.97–1.7</td>
<td>Benzodiazepines insensitive; furosemide and THIP sensitive</td>
<td>Sperk et al. (1997), McKernan and Whiting (1996), Brown et al. (2002), Peng et al. (2002), Farrant and Nusser (2005), Boehm et al. (2006), Mortensen et al. (2012)</td>
</tr>
<tr>
<td>a6β3δE</td>
<td>E</td>
<td>Cerebellum granule cells</td>
<td>0.076</td>
<td></td>
<td>Brown et al. (2002), Möhler et al. (2002), Mortensen et al. (2012)</td>
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<tr>
<td>a6β2/3δE</td>
<td>E</td>
<td>Account for ~2% of all GABA-A receptors. Cerebellum granule cells</td>
<td>0.17</td>
<td>Lacks benzodiazepine binding site; furosemide and THIP sensitive</td>
<td>Brown et al. (2002), Möhler et al. (2002), Mortensen et al. (2012)</td>
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<tr>
<td><strong>S/E</strong></td>
<td></td>
<td></td>
<td>1.4</td>
<td>Benzodiazepines and flumazenil sensitive</td>
<td>McKernan and Whiting (1996), Möhler et al. (2002), Farrant and Nusser (2005), Mortensen et al. (2012)</td>
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</table>

Benzodiazepines, GABA-A receptor allosteric agonists; E, extrasynaptic; flumazenil, a GABA-A receptor antagonist; furosemide, a GABA-A receptor antagonist; S, synaptic; zolpidem, a full agonist at α1-containing GABA-A receptors, ~10-fold lower affinity at α2- and α3-containing GABA-A receptors.
the receptor via several mechanisms, including the alteration of receptor functions, a reduction in receptor surface expression, and decreased receptor lifetime on the cell membrane.

A single base pair (bp) deletion (975delC) in GABRA1 was found in a patient with sporadic CAE among 98 unrelated patients with idiopathic generalized epilepsy (IGE), a generalized epilepsy with a strong underlying genetic basis. This de novo mutation causes a frameshift and a premature stop codon (S326fs328X), leading to a truncation in the TM3- and ER-associated degradation. In addition, GABA-evoked currents were not detected in the HEK293 cells with mutant α1-containing GABA-A receptors. These mutant receptors were not able to be incorporated into the membrane surface, highlighting the involvement of the α1 subunit in inserting the receptors into the cell membrane and the overall functional integrity of the GABA-A receptors (Maljevic et al., 2006). However, no dominant negative effects on the WT receptors were observed in these mutant receptors. These findings reveal that a single bp deletion in GABRA1 causes a loss of function and haploinsufficiency of the GABA-A receptors.

Two other mutations in GABRA1 associated with impaired membrane delivery of the mature GABA-A receptor were also identified in a cohort of French Canadian families with IGE. A 25-bp insertion associated with intron results in the deletion of the TM4 and a premature stop codon (K353delins18X), and a missense mutation that replaces the aspartate 219 residue with an asparagine (D219N). The family with K353delins18X mutation displays afebrile, generalized tonic-clonic seizures, whereas the family with the D219N mutation in GABRA1 shows mainly FSs and absence seizures (Lachance-Touchette et al., 2011). In addition, four novel de novo mutations in GABRA1 were also found in SCN1A-negative patients with DS, providing insight into more genetic causes for this syndrome (Carvill et al., 2014). Recently, several novel mutations in the extracellular N terminus of TM1 or TM2 of GABRA1 have been implicated in several epileptic diseases including infantile epilepsy, West syndrome, Ohtahara syndrome, and early onset epileptic encephalopathy (Johannesen et al., 2016; Kodera et al., 2016). Mutations in GABRA1 of GABA-A receptors contribute to the genetic etiology of both mild generalized epilepsies and severe epilepsy syndromes.

**Mutations of β3 Subunit.** The association of GABRB3 with idiopathic generalized epilepsies was first reported in the rodent brain (Fig. 3). Extrasynaptic GABA-A receptor δ subunit distribution. (A) The estimated abundance of GABA-A receptor subtypes in the rodent brain. (B) GABA-A receptor δ subunit distribution. *denotes δ subunit.

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### TABLE 4
Summary of GABA-A receptor genetic epilepsies
The positions of mutations are designated in the immature peptide including the 39 amino acid signal peptide.

<table>
<thead>
<tr>
<th>Genetic Location</th>
<th>Mutation</th>
<th>Sample Size</th>
<th>Receptor Dysfunction</th>
<th>Phenotype OMIM#</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CAE</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a1 (GABRA1)</td>
<td>5q34</td>
<td>(975delC, S326fs328X) A single bp deletion and premature stop codon in TM3</td>
<td>Sporadic 1 of 98 German patients with IGE</td>
<td>Reduced GABA current and surface expression (Maljevic et al., 2006)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1 of 72 patients with IGE, 65 patients with GEFS+, 66 patients with FS</td>
<td>Impaired receptor assembly/trafficking; reduced surface expression of δ subunit (Dibbens et al., 2009; Hernandez et al., 2011)</td>
</tr>
<tr>
<td>β3 (GABRB3)</td>
<td>15q12</td>
<td>Haploype 2 promoter in exon 1a</td>
<td>45 patients with CAE</td>
<td>Lower transcription activity (Feucht et al., 1999; Urak et al., 2006)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(P11S) A heterozygous missense mutation in exon 1a in signal peptide, causing the changes of proline to serine at position 11</td>
<td>4 of 48 patients with remitting CAE in a Mexican family</td>
<td>Reduced GABA current and increased glycosylation (Tanaka et al., 2008)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(S15F) A missense mutation in signal peptide, causing the changes of serine to phenylalanine at position 15</td>
<td>1 of 48 patients with remitting CAE from Honduras</td>
<td>Reduced GABA current and increased glycosylation (Tanaka et al., 2008)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(G32R) A missense mutation in exon 2 in N terminus, causing the changes of glycine to arginine at position 32</td>
<td>2 of 48 patients with remitting CAE in a Honduran family</td>
<td>Reduced GABA current and increased glycosylation (Tanaka et al., 2008)</td>
</tr>
<tr>
<td>γ2 (GABRG2)</td>
<td>5q34</td>
<td>(R52Q) A missense mutation in N terminus, causing the changes of arginine to glutamine at position 82</td>
<td>Autosomal dominant form in a large Australian family</td>
<td>Impaired receptor trafficking and reduced surface expression (Wallace et al., 2001)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(P11S) A heterozygous missense mutation in TM3, causing the changes of alanine to aspartate at position 32</td>
<td>14 members of a French Canadian family</td>
<td>Reduced GABA current and surface expression (Cossette et al., 2002)</td>
</tr>
<tr>
<td>δ (GABRD)</td>
<td>1p36.3</td>
<td>(R230H) A missense mutation in exon 6 in N terminus, causing the changes of glycine to histidine at position 220</td>
<td>A small GEFS+ family</td>
<td>Reduced surface expression and receptor mean open duration (Dibbens et al., 2004)</td>
</tr>
<tr>
<td><strong>FS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>γ2 (GABRG2)</td>
<td>5q34</td>
<td>(R52Q) A missense mutation in N terminus, causing the changes of arginine to glutamine at position 82</td>
<td>Autosomal dominant form in a large Australian family</td>
<td>Impaired receptor trafficking and reduced surface expression (Wallace et al., 2001)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(R177G) A missense mutation in N terminus, causing the changes of arginine to glycine at position 177</td>
<td>1 of 47 unrelated patients</td>
<td>Altered GABA current and kinetics and impaired subunit folding and/or oligomerization (Audenaert et al., 2006)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(K328M) A missense mutation in extracellular loop between the TM2 and TM3, causing the changes of lysine to methionine at position 328</td>
<td>A large French family</td>
<td>Altered GABA current and kinetics (Baulac et al., 2001)</td>
</tr>
<tr>
<td>GEFS+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>γ2 (GABRG2)</td>
<td>5q34</td>
<td>(K283M) A missense mutation in extracellular loop between the TM2 and TM3, causing the changes of lysine to methionine at position 328</td>
<td>A large French family with GEFS+</td>
<td>Altered GABA current and kinetics (Baulac et al., 2001)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(Q390X) A nonsense mutation in intracellular loop between the TM3 and TM4, causing a premature stop codon at position 390</td>
<td>A GEFS+ family</td>
<td>ER retention and abolished GABA sensitivity (Harkin et al., 2002; Kang et al., 2010)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(R136X) A missense mutation in N terminus, causing a premature stop codon at position 136</td>
<td>A two-generation family with GEFS+</td>
<td>Reduced receptor current amplitudes and surface expression, ER retention (Sun et al., 2008; Johnston et al., 2014)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(W429X) A nonsense mutation in intracellular loop between the TM3 and TM4, causing a premature stop codon at position 429</td>
<td>1 of 23 Chinese families with GEFS+</td>
<td>Undetermined, is predicted to translate a truncated protein (Sun et al., 2008; Johnston et al., 2014)</td>
</tr>
</tbody>
</table>

(continued)
1999 (Feucht et al., 1999). Urak et al. (2006) performed a mutation screening of GABRB3 of 45 patients with CAE and found 13 single nucleotide polymorphisms and 4 haplotypes in the haplotype 2 region of the GABRB3 promoter. Lowered transcriptional activity of this region is highly associated with CAE (Feucht et al., 1999; Urak et al., 2006). Thus, diminished expression of the GABRB3 gene could be a possible cause for the development of CAE.

Missense mutations (P11S, S15F, and G32R) in GABRB3 were found in families with remitting CAE (Tanaka et al., 2008). P11S and S15F are heterozygous mutations in the exon 1a that encodes the signal peptide of the GABRB3. The P11S mutation was found in four affected subjects of a two-generation Mexican family, and the S15F was found in one family from Honduras. G32R is a heterozygous mutation in the exon 2 of GABRB3 found in four affected persons of a two-generation Honduran family. Further investigation revealed that G32R mutation causes a partial shift of $\alpha_3\beta_3\gamma_2L$ receptors to $\alpha_3$ and $\beta_3$ receptors and a reduction in macroscopic current density (Gurba et al., 2012). HEK293T cells expressing GABA-A receptors with these mutations have reduced GABA-A receptor current density attributed to hyperglycosylation in the in vitro translation and translocation system. Results suggest that elevated glycosylation in the mutant exon 1a and exon 2 may contribute to CAE by interfering with the maturation and trafficking of GABA-A receptors and by affecting receptor function. Mutation screening performed in 183 French Canadian individuals with IGE, including 88 with CAE, also found nine single nucleotide polymorphisms and the P11S missense mutation in GABRB3 (Lachance-Touchette et al., 2010). However, another study demonstrated that no mutations in the exon 1a promoter of GABRB3 were found in 780 German patients with IGE, including 250 with CAE (Hempelmann et al., 2007). Therefore, the association of GABRB3 with CAE is still under debate.

**Mutations of $\gamma_2$ Subunit.** The $\gamma$-containing GABA-A receptors are the main mediators of fast inhibitory transmission through phasic current inhibition in the synaptic sites (Sieghart and Sperk, 2002; Farrant and Nusser, 2005; Olsen and Sieghart, 2009). The $\gamma$ subunit is required for the formation of the binding site for benzodiazepines. In addition, it is also important for the clustering of the GABA-A receptor subtypes in the postsynaptic sites (Essrich et al., 1998; Fang et al., 2006). Mutations in the gene encoding the $\gamma$ subunit of GABA-A receptors (GABRG) have been associated with the pathogenesis of epilepsy; mostly FS and GEFS+. The first evidence linking GABA-A receptor subunit genes with epilepsy was reported in 2001. GABRG2 was the first identified GABA-A receptor subunit gene that is involved in IGE (Baulac et al., 2001). More evidence was subsequently discovered supporting the association between genetic epilepsies and GABA-A receptor subunit genes. A missense mutation in GABRB2 found in a large French family with GEFS+ phenotype is caused by a substitution of a positively charged lysine residue for a neutral methionine (K328M) in the extracellular loop between the TM2 and TM3 of GABRG2. The recombinant receptors with this mutant $\gamma_2^{K328M}$ subunit expressed in Xenopus laevis oocytes have significantly lower amplitudes of GABA-evoked currents, indicating impaired receptor function (Baulac et al., 2001). Receptors with $\gamma_2^{K328M}$
subunit expressed in cultured rat hippocampal neurons displayed an accelerated decay time constant of miniature inhibitory post synaptic currents (mIPSCs). In addition, mutant γ2R136X receptors had decreased frequency of mIPSCs and enhanced membrane diffusion of receptors after a 1-hour exposure to elevated temperatures, which was not observed in WT receptors, suggesting a reduced number of functional inhibitory synapses and compromised GABAergic transmission (Bouthour et al., 2012). These could be a novel mechanism involved in the pathology of FS.

Two nonsense mutations (R136X and W429X) in GABRG2 were identified in a two-generation family with GEFS+ and in 1 of 23 Chinese families with GEFS+, respectively (Sun et al., 2008; Johnston et al., 2014). The R136X mutation in the GABRG2 reduces receptor current amplitudes and surface expression with greater intracellular retention when γ2R136X subunits were heterologously expressed in HEK293T cells with the α1 and β2 subunits since the mutant γ2R136X subunit did not allow the assembly of functional receptors. No dominant negative suppression effects from the mutant γ2R136X subunits on the WT receptors were noticed. The W429X is located in the extracellular loop between the TM3 and TM4 of GABRG2. It generates a premature translation termination codon and is predicted to translate a truncated protein.

A novel frameshift mutation in the GABRG2 was also identified in a family with GEFS+ (Tian et al., 2013). A cytotoxic nucleotide deletion in the last exon of GABRG2 results in a γ2S844delC subunit with a modified and elongated carboxy-terminus, γ2S844delC subunits, which are larger than WT γ2 subunits when translated, displayed higher retention in the ER and lower membrane expression. Electrophysiological characterization revealed significantly decreased peak GABA-evoked currents in HEK293T cells, showing GABRG2 haploinsufficiency.

Q390X, a nonsense mutation in the intracellular loop between the TM3 and TM4 of the GABRG2 was found in patients with GEFS+, FS, and DS. This mutation introduces a premature stop codon at Q390 in the immature protein. X. laevis oocytes expressing recombinant receptors with a mutant γ2Q390X subunit showed diminished response to GABA and retention in the intracellular compartment, which are also found in HEK293T cells expressing receptors with a mutant γ2R136X subunit (Harkin et al., 2002). Mutant γ2R136X subunits accumulated and formed high–molecular mass aggregation rapidly and showed longer half-life and slower degradation compared with WT subunits in several cell lines, including HEK293T, COS-7, and Hela cells, and in cultured rat cortical neurons (Kang et al., 2010). The accumulation and aggregation of misfolded or truncated proteins are commonly observed in neurodegenerative diseases. However, Kang et al. (2010) showed that the aggregates are also associated with genetic epilepsies. The GABrg2

A missense mutation (R82Q) in the GABRG2 was found in an Australian family and results in an autosomal dominant inherited form of CAE and FS (Wallace et al., 2001; Hancili et al., 2014). Arginine 82, located in the high-affinity benzodiazepine-binding region, abolishes benzodiazepine-potentiated currents in recombinant receptors expressed in X. laevis oocytes but does not affect Zn2+ sensitivity. Receptors with the mutant γ2 subunit (α3β3γ2R82Q) expressed in COS-7 cells display impaired receptor trafficking and reduced membrane expression due to impaired assembly into pentamers. Furthermore, ER retention and degradation leads to abnormal receptor function when α1β2γ2R82Q receptors were expressed in HEK293T cells (Wallace et al., 2001; Kang and Macdonald, 2004; Frugier et al., 2007; Huang et al., 2014). γ2R82Q receptors expressed in cultured rat hippocampal neurons and COS-7 cells show increased clathrin-mediated dynamin-dependent endocytosis, hindering their detection on the cell membrane (Chaumont et al., 2013). Animal studies revealed that γ2S844delC mice displayed enhanced cortical spontaneous single-cell activity, membrane potential shift, and variance of stimulus-evoked cortical responses after pentylenetetrazol injection, supporting the focus on the cortical region in the pathology of absence epilepsy (Tan et al., 2007; Witsch et al., 2015). A splice site mutation in intron 6 of the GABRG2 was also found in patients with idiopathic absence epilepsies, CAE, and FS. This mutation is predicted to translate a nonfunctional protein and may be involved in the pathophysiology of CAE and FS (Kanamura et al., 2002).

A missense mutation (R177G) in the GABRG2 was reported in patients with FS, GEFS+, and CAE (Audenaert et al., 2006). The mutation, located in the benzodiazepine allosteric site, substitutes a highly conserved arginine with glycine at position 177 in the immature peptide. Receptors with mutant γ2R177G subunit expressed in HEK293T cells conferred faster current desensitization and decreased sensitivity to diazepam. The disruption of the benzodiazepine binding site could be the reason for the diminished response to benzodiazepine drugs and abnormal receptor function and may contribute to the disinhibition of the brain. γ2L1R177G has been demonstrated to have decreased GABA sensitivity and surface expression due to the retention of receptors in the ER. γ2L1R177G subunits also showed impaired subunit folding and/or oligomerization and could interrupt intrasubunit salt bridges and, thereby, have destabilized secondary and tertiary structure (Todd et al., 2014).

A missense mutation (P83S) in the GABRG2 was found in patients with idiopathic generalized epilepsies in a French Canadian family (Lachance-Touchette et al., 2011). The mutation, which showed a high degree of penetrance, is caused by the change of the proline 83 residue of the immature protein to a serine in a region that affects benzodiazepine binding on the extracellular ligand-binding domain. Unlike other missense mutations found in the GABRG2 gene, functional analysis of the receptors revealed that γ2P83S expressed in HEK293 cells does not alter the surface expression or the receptor sensitivity to Zn2+ or benzodiazepine when coexpressed with α1 and β2 subunits. Further experiments are needed to elucidate whether an appreciable phenotype can be observed in the receptors with this mutant when assembling with other subunits.

A nonsense mutation (Q40X) in the GABRG2 was found in dizygotic twin girls with DS and their phenotypically healthy
father in a Japanese family (Ishii et al., 2014). The mutation causes premature termination codons at position 40 of the GABRG2 molecule. The inward currents and current density of receptors with homozygous γ2Q40X mutation expressed in HEK293T cells in response to GABA were intermediate between WT and heterozygous α1β2γ2Q40X GABA-A receptors, showing the haploinsufficiency effects of mutant γ2Q40X. In addition, cells expressing this mutant subunit have abnormal intracellular trafficking and impaired axonal transport of α1 and β2 subunits.

**Mutations of δ Subunit.** The δ-containing GABA-A receptors primarily control the baseline neuronal network excitability through shunting and tonic inhibition. Mutations in the GABRD are thought to be involved in the pathology of epilepsy (Dibbens et al., 2004; Feng et al., 2006). To date, three point mutations (E177A, R220C, and R220H) of GABRD have been described in patients with idiopathic generalized epilepsies (a cohort study with 72 unrelated patients with IGE, 65 unrelated patients with GEFS+ family (Dibbens et al., 2004). E177 is located immediately adjacent to one of the two cysteines that form a disulfide bond, the signature feature of the cys-loop family of ligand-gated ion channels, and the R220 is positioned between the cys-loop and the first TM. In HEK293T cells, recombinant α1δ258 GABA-A receptors expressing E177A and R220H mutations displayed decreased GABAA sensitivity and increased neuronal excitability. Further investigation revealed that human recombinant α4β2δ GABA-A receptors with E177A and R220H variants have significantly reduced receptor surface expression and altered channel gating frequency, resulting in impaired inhibitory neurotransmission. In addition, mutations in the main cytoplasmic loop of the δ subunit result in interrupted receptor trafficking and decreased surface expression in recombinant HEK293 cells (Bracamontes et al., 2014). Since δ-containing GABA-A receptors localize exclusively to extrasynaptic membranes and control tonic inhibition by continuously regulating the basal tone of inhibition when activated by GABA, alteration of features such as gating frequency or GABA sensitivity caused by mutations in δ subunit genes may contribute to the idiopathic generalized epilepsies.

**Other GABA Receptor Subunit Mutations.** A mutation in GABRA6 (R46W) was found in a patient with CAE (Dibbens et al., 2009; Hernandez et al., 2011). This mutation, caused by the substitution of arginine for tryptophan at position 46, is located in the N-terminal extracellular domain of the α6 subunit. Notably, R46 is a highly conserved residue identified from humans to *Drosophila melanogaster* and *Caenorhabditis elegans*. R46W impaired receptor assembly/trafficking and gating in both α6β2γ2L receptors (Carter et al., 1997). These results suggest that mutations in GABRA6 could lead to disassembly and elevated susceptibility to genetic epilepsies due to compromised receptor function and membrane expression.

In the past decades, no genetic studies demonstrated that mutations in GABRB2 are associated with genetic epilepsies. Until recently, Srivastava et al. (2014) reported a case study in which a de novo heterozygous missense mutation of GABRB2 was found in a girl with intellectual disability and epilepsy. Their finding suggests the need for further investigation into the role of GABRB2 in the pathology of epilepsy and other neurologic disorders.

**Neuroendocrine Regulation of Extrasynaptic GABA-A Receptors**

**NS Modulation of GABA-A Receptors and Tonic Inhibition.** NSs, also referred to as neuroactive steroids, are steroids with rapid actions on neuronal excitability through the modulation of membrane receptors in the CNS (Kulkarni and Reddy, 1995; Mellon and Griffin, 2002). The terminology for “neurosteroid” and “neuroactive steroid” has been described extensively in the literature (Reddy and Rogawski, 2012). Generally, the terms NSs and neuroactive steroids are used interchangeably to refer to endogenous steroids with rapid membrane actions that were either made de novo in the brain or from peripheral sources of parent steroids that were metabolized in the brain (Baulieu, 1981; Paul and Purdy, 1992; Reddy, 2003a,b, 2009a, 2011, 2013b). Parent steroids or NS precursors (not the steroid metabolites) bind to classic steroid receptors. A brief outline of NS synthesis is described below. Cholesterol is first translocated across the mitochondrial membrane by TSP0 (translocator protein 18 kDa, a key protein that controls the biosynthesis of NSs) and converted into steroid precursor pregnenolone by the cytochrome P450 side-chain cleavage enzyme in the inner membrane of mitochondria. Pregnenolone is subsequently converted into NS precursors progesterone, deoxycorticosterone, and testosterone, which go through two sequential A-ring reduction steps and are converted into three prototype endogenous NSs [allopregnanolone (AP; 3α-hydroxy-5α-pregnan-20-one), allotetrahydroxydeoxycorticosterone (THDOC), and androstanediol)] by the catalysis of two key enzymes called 5α-reductase and 3α-hydroxysteroid oxidoreductase in the brain. These three prototype NSs, AP, THDOC, and androstanediol, have been well studied (Reddy, 2003a, 2009a, 2011; Rupprecht, 2003; Belelli and Lambert, 2005; Carver and Reddy, 2013; Brown et al., 2015; Porcu et al., 2016). THDOC potentiates both tonic current conductance and the weighted decay time of spontaneous IPSCs in directly in DGGCs and cerebellar granule cells (Carter et al., 2002). THDOC at low concentrations (30 and 100 nM) potentiates the decay time of mIPSCs in CA1 pyramidal cells (Carter et al., 2002). THDOC at low concentrations (30 and 100 nM) potentiates the decay time of mIPSCs in CA1 pyramidal cells (Carter et al., 2002). THDOC potentiates both tonic current conductance and the weighted decay time of spontaneous IPSCs directly in DGGCs and cerebellar granule cells (Carter et al., 2002). THDOC potentiates both tonic current conductance and the weighted decay time of spontaneous IPSCs directly in DGGCs and cerebellar granule cells (Carter et al., 2002). THDOC potentiates both tonic current conductance and the weighted decay time of spontaneous IPSCs directly in DGGCs and cerebellar granule cells (Carter et al., 2002).
seizure models (Reddy and Rogawski, 2000a,b, 2010). GX is currently being evaluated in clinical trials for the treatment of epilepsy and related conditions (Nohria and Giller, 2007; Reddy and Rogawski, 2010, 2012; Bialer et al., 2015; Braat et al., 2015; Ligsay et al., 2017).

Progesterone, GX, and AP have been evaluated in clinical trials for seizure conditions (Reddy and Estes, 2016; Younus and Reddy, 2017). GX has been evaluated in more than 1300 subjects in various clinical studies in adults and children with epilepsy (Kerrigan et al., 2000; Laxer et al., 2000; Reddy and Woodward, 2004; Nohria and Giller, 2007; Pieribone et al., 2007; Sperling et al., 2017). Progesterone was evaluated as an adjunct therapy in women with epilepsy (Herzog et al., 2012). The NS AP (brexanolone) has been suggested as an intravenous therapy for refractory SE (Rosenthal et al., 2017) and postpartum depression (Kanes et al., 2017). Overall, synthetic NS analogs, which activate both synaptic and extrasynaptic GABA-A receptor–mediated tonic inhibition in the brain, may be promising compounds for the clinical development of specific seizure conditions, such as status epilepticus and catamenial epilepsy.

**Protein Kinase Modulation of NS-Sensitive GABA-A Receptors.** Protein kinases are enzymes that can regulate the function of other proteins by phosphorylating hydroxyl groups on the proteins by which they act. Protein kinase activity influences GABA-A receptor surface expression, trafficking, chloride conductance, and sensitivity to NSs. Several GABA-A receptor subunits contain residues that can be phosphorylated by protein kinases including α4, β, and γ2 subunits (Moss and Smart, 1996; Brandon et al., 2000). The serine residue (Ser-443) of the δ4 subunit is phosphorylated by protein kinase C (PKC) (Abramian et al., 2010). A conserved serine residue (Ser-409 or Ser-410) of the β subunit is phosphorylated by PKC, protein kinase A (PKA), Ca2+/calmodulin-dependent protein kinase II (CaMKII), and cGMP-dependent protein kinase. Two additional serine residues of the β subunit (Ser-408 and Ser-383) are also phosphorylated by PKA and CaMKII, respectively (McDonald et al., 1998; Saliba et al., 2012). Additionally, the tyrosine residues (Tyr-365 and Tyr-367) and serine residue (Ser-343) of the γ2 subunit are also substrates of tyrosine kinase and PKC, respectively (Krishek et al., 1994; Moss et al., 1995). Phosphorylation of residues within the intracellular loops of the β3 and γ2 subunits maintains the surface expression of GABA-A receptors, whereas, dephosphorylation of these subunits triggers receptor internalization (Kittler et al., 2005, 2008) (Fig. 5).

Previous studies have shown that PKC activation regulates receptor function, ion conductance, and ion response to receptor modulators. Ten-minute bath application of PKC activator phorbol 12-myristate 13-acetate increases THDOC-potentiated, GABA-gated chloride currents (Leidenheimer and Chapell, 1997). Treatment with the specific PKC antagonist bisindolylmaleimide 15 minutes before and during NS administration diminishes the decay of IPSCs by NSs (Fáncsik et al., 2000). The inhibition of either PKA or PKC by intracellular dialysis significantly reduces NS 5β-pregn-3a-ol-20-one–mediated prolonged decay of mIPSCs in CA1 pyramidal neurons, whereas the activation of PKC has no effect on NS sensitivity (Harney et al., 2003). Phosphorylation of the serine residue of the β subunits (Ser-383) by L-type voltage-gated Ca2+ channel–activated CaMKII leads to rapid surface expression of GABA-A receptors and enhanced tonic currents in hippocampal neurons (Saliba et al., 2012). Ten-minute cotreatment with PKC inhibitor prevents THDOC-upregulated phosphorylation of the α4 subunits and surface expression of α4-containing GABA-A receptors (Abramian et al., 2014). A recent study (Modgil et al., 2017) demonstrated that continuous application of AP, but not GX, upregulates the phosphorylation and surface expression of the β3-containing GABA-A receptors and tonic current potentiation, effects prevented by the application of PKC inhibitor 15 minutes before and during NS application.

**Zinc Antagonism of NS-Sensitive GABA-A Receptors.** Zinc (Zn2+) is an essential cofactor in many cells including neurons. Zn2+ is the most abundant transition metal in the vesicles of hippocampal mossy fibers that project from the DG to the CA3 (Frederickson, 1989). During neuronal activity, vesicular Zn2+ is released synaptically from certain nerve terminals in the hippocampus (Tian et al., 2010). Excessive release of Zn2+ has been shown in epilepsy, and it can decrease the threshold of excitability and seizures (Takeda et al., 1999; Coulter, 2000; Foresti et al., 2008). Zn2+ regulation of postsynaptic targets and synaptic plasticity is shown in Fig. 6. Zn2+ modulates several ligand- and voltage-gated ion channels (Harrison and Gibbons 1994). In particular, Zn2+ attenuates GABAergic inhibition at mossy fiber synaptic varicosities that release GABA (Xie and Smart, 1991; Ruiz et al., 2004; Bitanihirwe and Cunningham, 2009). In addition, Zn2+ has been shown to negatively modulate synaptic GABA-A receptors and modify the excitability of the hippocampal network (Barberis et al., 2000). The following three distinct Zn2+ binding sites mediate its inhibition of GABA-A receptors: one site at the internal surface of the channel pore and two at the external amino terminus of the α-β interfaces. The incorporation of the γ subunit after GABA-A receptor coassembly disrupts two of the Zn2+ binding sites, which leads to a reduced sensitivity to Zn2+ inhibition ( Hosie et al., 2003). Thus, the sensitivity to Zn2+ inhibition is different between the two different GABA-A receptor subtypes (δ containing and γ containing) (Smart et al., 1991; Störstrup and Ebert, 2006).

Our recent study demonstrated the δ-containing receptors to be more sensitive to Zn2+ inhibition than γ-containing receptors, and Zn2+ selectively blocks NS-sensitive extrasynaptic δGABA-A receptor–mediated tonic currents in the mouse hippocampus dentate gyrus (Carver et al., 2016). Zn2+ blocked AP potentiation of tonic currents in a concentration-dependent manner, whereas synaptic currents were unaffected. Application of Zn2+ chelator prevented the positive shift of tonic currents by Zn2+, confirming the Zn2+ blockade of AP-sensitive tonic currents. In the mouse kindling model of epilepsy, intrahippocampal infusion of Zn2+ resulted in rapid epileptiform activity and the prevention of the antiseizure activity of AP. Therefore, Zn2+ inhibition of NS-potentiated, extrasynaptic GABA-A receptors in the hippocampus has direct involvement in a variety of brain conditions, such as seizures, epileptogenesis, epilepsy, and conditions with compromised balance of excitation/inhibition. Both Zn2+ and NSs show high affinity for extrasynaptic δ-containing GABA-A receptors, but their actions are distinctly opposite through the binding of different allosteric sites. Overall, Zn2+ hinders NS activation of extrasynaptic δGABA-A receptor–mediated tonic inhibition and their ability to promote neuroprotection and inhibit seizure activity in the brain.
Insights from Transgenic Models on Tonic Inhibition. To elucidate the distinctive pharmacological and functional properties and the role of the δ subunit in brain disorders, a strain of mice globally lacking the δ subunit [GABA-A receptor δ subunit knockout (DKO)] of GABA-A receptors was introduced in 1999 (Mihalek et al., 1999). Initial pharmacological and behavioral characterizations revealed the DKO mice have significantly lower binding affinity for muscimol, a GABA binding agonist with high affinity to δ-containing GABA-A receptors, and a faster decay of mIPSCs and inhibitory postsynaptic potentials than that of WT mice. In addition, DKO mice show significantly decreased sleep time in response to alphaxolone, a NS and general anesthetic, diminished sensitivity to anxiolytic drugs examined by elevated plus-maze assay, diminished GX-facilitated exacerbation of pentylenetetrazol-induced absence seizure, and higher vulnerability to the chemoconvulsants seizures caused by GABA-A receptor antagonists. Their results suggest that deficiency of the δ subunit of the GABA-A receptors leads to a significant attenuation in the sensitivity to behavioral actions of NSs, which may contribute to a higher degree of seizure susceptibility (Mihalek et al., 1999; Spigelman et al., 2002, 2003; Porcello et al., 2003; Chandra et al., 2010).
The δ-specific selectivity for NS modulation is further confirmed by electrophysiological studies demonstrating suppressed responses to THDOC modulation such as decreased spontaneous IPSCs in cerebellar granule cells and decreased tonic conductance in DGGCs in the DKO mouse model (Vicini et al., 2002; Wohlfarth et al., 2002; Stell et al., 2003). In addition, attenuated response to AP potentiation of GABAergic and tonic currents is also evident in mice bearing a targeted deletion of the δ subunit, underscoring the role of δ-containing GABA-A receptors for NS activity (Carver et al., 2014; Carver and Reddy, 2016). Reduced response to gaboxadol (THIP)-induced hypnotic activity is also reported in δ-deficient mice, suggesting the requirement of δ-containing GABA-A receptors in the action of THIP, a preferential agonist of δ-containing GABA-A receptors (NMDA Rs), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPA Rs), voltage-gated calcium channels (VGCCs), GABA-ARs, and a number of other channels, transporters, and receptors. Three distinct Zn²⁺ binding sites mediate its inhibition of extrasynaptic δ-containing GABA-ARs (inset, right): one site at the internal surface of the channel pore and two at the external amino terminus of the α-β interfaces.

Therapeutic Insights of Tonic Inhibition in Epilepsy

Extrasynaptic GABA-A receptors are involved in the pathophysiology of certain brain conditions, such as catamenial
epilepsy, SE, and other neuroendocrine disorders (Belelli and Lambert 2005; Brickley and Mody, 2012; Reddy, 2013a,b, 2016). Consequently, these receptors are emerging as novel targets for excitability disorders (Reddy and Estes, 2016). Substantial evidence suggests that NS-sensitive, extrasynaptic GABA-A receptors play a critical role in the pathophysiology of catamenial epilepsy, a menstrual cycle–related seizure clustering in women with epilepsy (Reddy et al., 2001, 2012; Reddy and Rogawski, 2001, 2012; Reddy, 2009a, 2014, 2016a, 2017; Wu et al., 2013). Although this condition has been documented for millennia, there is currently no effective treatment of catamenial seizures, leaving many women and their families desperate for answers. Recently, a catamenial-like seizure exacerbation has been clearly demonstrated in mice with targeted ablation of extrasynaptic δGABA-A receptors in the brain (Clossen and Reddy, 2017). This has substantially bolstered the role of tonic inhibition in catamenial epilepsy (Reddy, 2016a). In essence, extrasynaptic δGABA-A receptors are strikingly upregulated during perimenstrual-like neuroendocrine milieu (Gangisetti and Reddy, 2010; Carver et al., 2014). Consequently, there is greater antiseizure efficacy of NSs in catamenial models because δGABA-A receptors confer enhanced NS sensitivity and greater seizure protection. Therefore, this molecular mechanism of tonic inhibition as the major regulator of the catamenial seizures is providing a strong platform for “neurosteroid replacement therapy,” a pulse therapy with low doses of synthetic NS agents that may effectively control catamenial seizures without hormonal side effects (Reddy and Rogawski, 2009).

NS levels are reduced during SE, a neurologic emergency characterized by continuous seizure activity or multiple seizures without regaining consciousness for more than 30 minutes (Meletti et al., 2017). Benzodiazepines such as lorazepam and midazolam are the primary anticonvulsants for SE, but some patients do not respond to these treatments, a condition referred to as refractory SE (Reddy and Reddy, 2015). Benzodiazepines target synaptic GABA-A receptors but have little effect on extrasynaptic isoforms, which are responsible for tonic inhibition (Reddy et al., 2015). There are many theories, but functional inactivation of synaptic GABA-A receptors via active internalization appears to be a lead physiologic mechanism by which benzodiazepine resistance emerges in SE (Naylor et al., 2005; Reddy and Reddy, 2015). Therefore, NS agents such as AP and its synthetic analogs, which potentiate both phasic and tonic current, have been proposed as better anticonvulsant agents for the treatment of SE (Briyal and Reddy, 2008; Reddy, 2009b; Rogawski et al., 2013). Clinical evaluation of AP is in progress to test this therapeutic premise (Rosenthal et al., 2017; Vaitkevicius et al., 2017).

Synthetic NSs are proposed as novel anticonvulsant antioxidants for chemical intoxication caused by organophosphate (OP) pesticides and nerve agents like sarin and soman (Reddy, 2016b). Benzodiazepines, such as diazepam, are the current anticonvulsants of choice for controlling nerve agent–induced seizures, SE, and brain injury. Benzodiazepines can control acute seizures when given early, but they are ineffective for delayed treatment of SE after nerve agent exposure (Reddy and Reddy, 2015). NS-sensitive extrasynaptic GABA-A receptors are minimally impacted by OP intoxication. Thus, anticonvulsant NSs may produce more effective protection than benzodiazepines against a broad spectrum of chemical agents, even when given late after nerve agent exposure, because NSs can activate both synaptic and extrasynaptic GABA-A receptors and thereby can produce maximal inhibition (Carver and Reddy, 2016). An intramuscular GX product is being developed as an anticonvulsant antidote for nerve agents. A NS therapy with a synthetic NS such as GX has been found to more protective than midazolam for controlling persistent SE and neuronal damage caused by OP pesticides and nerve agents (Reddy, 2016b). Although NSs show great promise in the treatment of SE, none are currently approved by the Food and Drug Administration for clinical use.

Some endogenous NSs, such as dehydroepiandrosterone sulfate (DHEAS), are negative modulators of GABA-A receptors and can cause proconvulsant actions (Majewska et al., 1990; Reddy and Kulkarni, 1998). It is likely that such actions have clinical implications in certain physiologic and pathologic conditions (Galimberti et al., 2005; Reddy, 2006, 2009a,b; Pack et al., 2011). It is hypothesized that the increase in the frequency of onset of seizures during the process of adrenarche, which is associated with a massive increase in DHEAS production, is caused by the ability of DHEAS to block GABA-A receptors. However, it remains unclear whether DHEAS can negatively modulate extrasynaptic GABA-A receptors in the brain. In addition, there are developmental changes in hippocampal extrasynaptic GABA-A receptors during puberty with significant impact on cognitive function (Shen et al., 2010; Reddy, 2014).

Conclusions and Perspectives

Recent advances in our knowledge of GABA-A receptor genetics provide great opportunities to further explore the underlying mechanisms of epileptogenesis and to discover effective interventions for the prevention, control, and cure of epilepsy. By comprehensively classifying and mapping the relevance of genetic epilepsies and gene mutations, the roles of GABA-A receptor function and subunit composition in the pathophysiology of epilepsy can be elucidated. In combination with advancing our understanding of the interactions between NSs and GABA-A receptors and their impacts on inhibitory neuronal transmission, more promising therapeutic implications may be disclosed. Factors that result in the imbalance of inhibition and excitation in the brain are associated with the occurrence of epilepsy. Specific GABAergic genetic aberrations lead to some of the classic epileptic syndromes. Dysregulation of neuronal activity and changes in the composition and function of GABA-A receptors contribute to the development of epilepsy.

Mutations in δ subunit are found in patients with genetic epilepsies. Mice lacking the δ subunit of GABA-A receptors have attenuated sensitivity to NSs in behavioral outcomes and electrophysiological characterizations, contributing to higher seizure vulnerability. Extrasynaptic δ-containing GABA-A receptors produce tonic currents to maintain the baseline of inhibition in the brain when activated by specific agonists. NSs interact with extrasynaptic GABA-A receptors and potentiate tonic inhibition through multiple mechanisms, providing potential therapeutics for hyperexcitable brain disorders such as SE, epileptogenesis, and epilepsy. The clinical trials of NSs for various conditions has been described previously (Reddy and Estes, 2016). The discovery of an extrasynaptic mechanism is providing a strong platform for novel therapies such as NS replacement therapy for catamenial epilepsy (Reddy, 2016a).
NSs have been proposed as more effective anticonvulsants than benzodiazepines for controlling refractory seizures, as persistent SE, that occur after OP intoxication and nerve agent exposure (Reddy, 2016b). Future work will certainly consolidate our knowledge in genetic epilepsies and facilitate the improvement of selective interventions for modulating the neuronal network under physiologic and pathologic conditions.

**Highlights**

- GABA-A receptors play a critical role in epilepsy and many brain disorders.
- Extrasympathetic GABA-A receptors are intrinsically involved in regulating the network excitability and behavior.
- Mutations in δGABA-A receptors lead to diminished tonic inhibition and epileptic seizures.
- Neuroendocrine control of extrasympathetic GABA-A receptors provides additional regulation of tonic inhibition in the brain.
- The tonic inhibition is a unique new mechanism in epilepsy, status epilepticus, and certain brain disorders.

**Authorship Contributions**

Wrote or contributed to the writing of the manuscript: Chuang, Reddy.

**References**


dominant juvenile myoclonic epilepsy reduces the expression and alters the composition of wild-type GABA(A) receptors. J Biol Chem 283:28280–28245.


