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Functional regulation of GABA_A receptors in nervous system pathologies

Rochelle M Hines¹, Paul A Davies¹, Stephen J Moss^{1,2}, and Jamie Maguire¹

¹Department of Neuroscience, Tufts University School of Medicine, 136 Harrison Ave, Boston, MA 02111, USA

²Department of Neuroscience, Physiology and Pharmacology, University College London, London WC1E 6BT, UK

Abstract

Inhibitory neurotransmission is primarily governed by γ -aminobutyric acid (GABA) type A receptors (GABA_ARs). GABA_ARs are heteropentameric ligand-gated channels formed by the combination of 19 possible subunits. GABA_AR subunits are subject to multiple types of regulation, impacting the localization, properties, and function of assembled receptors. GABA_ARs mediate both phasic (synaptic) and tonic (extrasynaptic) inhibition. While the regulatory mechanisms governing synaptic receptors have begun to be defined, little is known about the regulation of extrasynaptic receptors. We examine the contributions of GABA_ARs to the pathogenesis of neurodevelopmental disorders, schizophrenia, depression, epilepsy, and stroke, with particular focus on extrasynaptic GABA_ARs. We suggest that extrasynaptic GABA_ARs are attractive targets for the treatment of these disorders, and that research should be focused on delineating the mechanisms that regulate extrasynaptic GABA_ARs, promoting new therapeutic approaches.

Introduction

Fast inhibition in the central nervous system (CNS) is governed by the actions of γ -aminobutyric acid (GABA) type A receptors (GABA_ARs). GABA_ARs mediate both phasic and tonic inhibition, and are the principle sites of action for numerous classes of drugs including anxiolytics and anticonvulsants, as well as sedative hypnotic agents including benzodiazepines, barbiturates, alcohol, some general anesthetics and neurosteroids. GABA_ARs are ubiquitously expressed throughout the CNS, and as such, changes in their expression and function are implicated in virtually all aspects of brain function. Deficits in GABA_AR-mediated neurotransmission are implicated in diverse disorders of the CNS such as epilepsy [1], anxiety disorders [2], mood disorders [3,4], and neurodevelopmental disorders such as autism [5], Fragile X [6], and schizophrenia [7,8].

GABA_ARs are heteropentameric GABA-gated chloride channels that belong to the Cys-loop ligand-gated ion channel superfamily. To date, 19 subunits have been cloned in the mammalian CNS, divided into classes based on sequence identity (~70% identity within class, 30–40% between classes): α (1–6), β (1–3), γ (1–3), δ , ϵ , π , θ , and ρ (1–3). The most divergent portion of the sequence among GABA_AR subunits is the cytoplasmic loop lying between TM3 and TM4. This divergent portion is the site for a number of posttranslational modifications mediating both the channel function and the interaction of subunits with scaffolding proteins involved in regulating trafficking and localization. Each of the subunits

has distinct regional, cellular, and even subcellular distribution patterns achieved through numerous regulatory mechanisms, and it is well established that subunit combination and localization determines the biophysical and pharmacological function of the assembled GABA_AR.

Control of GABA_AR function

GABA_ARs comprising $\gamma 2$ and $\alpha 1-3, \beta x, \gamma 2$ are the most common type of receptor at synaptic sites and as such are primarily responsible for mediating phasic inhibition (Figure 1). GABA_ARs enriched at extrasynaptic sites, responsible for mediating tonic inhibition, are comprised primarily of $\alpha 4$ and $\alpha 6$ subunits combined with the δ subunit ($\alpha 4/6, \beta x, \delta$; Figure 1) [9]. Interestingly, GABA_ARs containing $\alpha 5$ can be localized to both synaptic and extrasynaptic sites, and as such contribute to both phasic and tonic inhibition [10].

In addition to subunit composition and localization, further mechanisms exist that control GABA_ARs on a rapid time-scale, such as regulation of receptor trafficking, clustering, and surface expression (reviewed in [11,12]). As is known for many types of receptors, GABA_ARs are not constitutively expressed at the cell surface, but cycle between the membrane and intracellular compartments via regulated exocytosis and endocytosis. A number of key proteins involved in regulating the cycling of GABA_ARs between the surface and intracellular pools have been identified, including GABA receptor associated protein (GABARAP) [13], protein linking integrin-associated protein to cytoskeleton-1 (Plic-1) [14,15], and clathrin adaptor protein 2 (AP2) [16]. Studies have shown that GABA_ARs are inserted into and removed from the membrane exclusively at extrasynaptic sites, and then require regulated lateral movement to be enriched at synaptic sites [17,18].

Selective blockade of synaptic GABA_ARs, via mutation of the $\alpha 1$ subunit rendering it sensitive to a use-dependent blocker, has shown that the synaptic receptor population can recover within minutes by importing of functional receptors via diffusion from extrasynaptic sites [18]. In addition, it has been shown that the diffusion dynamics of GABA_ARs are modified by excitatory neuronal activity. Specifically, excitatory synaptic activity reduces the stability of GABA_AR clusters at synapses in a calcium-dependent manner [19,20], resulting in enhanced translocation of receptors to extrasynaptic portions of the membrane during periods of high excitatory synaptic activity.

The clustering and accumulation of GABA_ARs at synaptic sites relies upon the interaction of multiple receptor-associated, adhesion, and scaffold proteins, including gephyrin [21,22], collybistin [25], neuroligin2 [26,27], neuroligin [28], the dystrophin-glycoprotein complex [29], and radixin [30] (Figure 1). Despite a common pool of interacting proteins, heterogeneity exists in the complex mechanisms governing clustering of receptors containing different subunits, likely dependent upon the combination of interacting proteins involved, and their relative interaction affinities.

In addition, GABA_AR localization and function are known to be regulated by the lipid modification palmitoylation (via Golgi-specific DHHC (Asp-His-His-Cys) zinc finger protein (GODZ); [31]), ubiquitination [32], and phosphorylation sites that have been identified and characterized on multiple subunits [33,34,35]. While the complex mechanisms governing localization of GABA_ARs at synaptic sites have been somewhat clarified, very little is known about the mechanisms that are involved in regulation of extrasynaptic GABA_AR localization.

GABA_ARs in neurological disorders

It is established that genetic mutations in GABA_ARs play a role in multiple neurological and neuropsychiatric disorders, which has been the subject of recent reviews [36,37]. Therefore, the present review will focus on posttranslational regulation of GABA_ARs in neurological disorders, specifically highlighting the implications of extrasynaptic GABA_ARs in disease. Insights into the mechanisms regulating extrasynaptic GABA_ARs may provide significant therapeutic potential for numerous diseases in which they have been implicated, including depression, epilepsy, neurodevelopmental disorders, schizophrenia, and stroke.

Neurodevelopmental disorders

Most neurodevelopmental disorders have overlapping symptoms such as anxiety, sleep dysfunction, cognitive deficits, and epilepsy. This common set of features has been linked to a decreased inhibitory tone leading to an excitatory/inhibitory (E/I) imbalance [38]. Autism, Angelman, Rett, and Fragile X syndromes all have a GABAergic hypofunction component. Conversely, Down syndrome is associated with GABA_AR hyperfunction (reduced E/I ratio) as recent animal model studies have shown the reversal of the syndrome's associated cognitive defects with an antagonist at GABA_ARs and $\alpha 5$ subunit inverse agonist [39*,40].

Postmortem studies from subjects with autism show a reduced level of [³H]-muscimol binding compared with control [41]. Muscimol has a higher affinity for $\alpha 4/\delta$ -containing receptors than other GABA_AR [42]. Indeed, muscimol binding is significantly reduced in δ subunit knockout (KO) mice [43]. Recently, expression levels of subunits were found to be significantly reduced in different regions of brains from autistic subjects compared with age-matched controls. Protein expression of $\alpha 1$, $\alpha 2$, $\alpha 3$, $\alpha 5$, and $\beta 3$ subunits were reduced in the parietal cortex whereas in the frontal cortex $\alpha 1$, $\alpha 4$, $\alpha 5$, and $\beta 1$ subunits were reduced [44,45]. These data would suggest a GABA-ergic hypofunction and in particular, a reduced tonic inhibition of patients with autism.

Similar information on subunit expression in humans is limited with other syndromes; however, animal models of syndromes further indicate GABAergic dysfunction and in particular, reductions in GABA_AR-mediated tonic inhibition as contributors to the symptoms. The Fragile X mental retardation 1 (fmr1) KO mice are used to model Fragile X syndrome. Studies have shown that in fmr1 KO mice mRNA encoding for $\alpha 1$, $\alpha 3$, $\alpha 4$, $\beta 1$, $\beta 2$, $\gamma 1$, $\gamma 2$, and δ subunits were reduced in cortex (and hippocampus for the δ subunit) but not cerebellum [6,46]. At the protein level, reductions in $\alpha 5$ and δ subunits [47*] and a reduction in an unspecified β subunit [48] have also been noted in a Fragile X mouse model. Electrophysiological recordings from the amygdala of fmr1 KO mice show a hyperexcitability in principle neurons because of a reduced frequency and amplitude of phasic IPSCs and a reduced tonic inhibitory current [49**]. In support of the therapeutic potential of targeting extrasynaptic GABA_ARs in neurodevelopmental disorders, the authors used THIP, an agonist at $\alpha 4/\delta$ subunit-containing receptors to reverse the hyperexcitability.

Schizophrenia

Since the early 1970s, GABAergic deficits have been proposed to play a role in the pathophysiology of schizophrenia [50]. In addition to alterations in synaptic receptors [51], alterations in extrasynaptic GABA_ARs have also been implicated in schizophrenia (for review see [52]), including decreased expression of several GABA_AR subunits in the dorsolateral prefrontal cortex (PFC), including the δ subunit [53]. Postmortem studies also reveal an increase in GABA_AR $\alpha 5$ subunit expression [54,55]. It has been proposed that these alterations in GABA_ARs are a compensatory change because of deficits in GABAergic neuro-transmission, consistent with decreased GABA levels [56] and GAD67 expression, in

schizophrenia [52]. However, GABA agonists have failed to be therapeutic in the treatment of schizophrenia, resulting in unwanted side effects including psychosis, sedation, amnesia, tolerance, dependence, confusion, and disorientation [52]. Thus, targeting extrasynaptic GABA_ARs may be a beneficial therapeutic target for the treatment of schizophrenia.

In animal models, GABA_AR antagonism in the PFC induces impulsivity [57] and cognitive impairments [58*]. Specifically, deficits in the extrasynaptic GABA_AR $\alpha 5$ subunit have been implicated in schizophrenia [59]. Mouse models deficient in the GABA_AR $\alpha 5$ subunit have abnormalities in prepulse inhibition [60], indicative of deficits in sensorimotor gating consistent with a schizophrenia-like phenotype [61]. Targeting extrasynaptic GABA_ARs reverses recognition memory deficits in an animal model of schizophrenia [62]. In addition, a novel allosteric modulator of GABA_AR $\alpha 5$ subunit-containing receptors, SH-053-2'F-R-CH₃, has been shown to be therapeutic in the methylazoxymethanol acetate developmental model of schizophrenia [63*].

Depression

The role of GABA_ARs in depression has generated significant interest because of the numerous compounds exhibiting antidepressant/mood-stabilizing effects which have effects on GABAergic transmission, including, tricyclic antidepressants, selective serotonin uptake inhibitors valproate, vigabatrin, gabapentin, tiagabine, benzodiazepines, alcohol, and neurosteroids (for review see [64]). Deficits in GABAergic inhibition have been proposed to play a critical role in the pathophysiology of depression (for reviews see [4,64]). This hypothesis is supported by studies demonstrating reductions in cortical GABA levels in patients suffering with depression [65], which are restored to normal levels with antidepressant treatment [65]. Postmortem studies demonstrate alterations in numerous GABA_AR subunit genes in patients with depression [66,67,68,69,70], such as a decrease in the expression of GABA_AR $\alpha 1$, $\alpha 3$, $\alpha 4$ [66,67,68], $\beta 3$ [68,69], and $\gamma 1$ [68,70] subunits; whereas, there is an increase in the synaptically localized GABA_AR $\gamma 2$ subunit [68,70]. In addition, an increase in the expression of the extrasynaptic $\alpha 5$ [68,70] and δ [66,68,69,70] subunits has also been associated with depression. Consistent with a role of GABA_AR deficits in depression, data from experimental models demonstrate depression-like behavior in mouse models with subtype-specific deficits in GABA_ARs, including *Gabrg*^{+/-} mice [71,72**] and *Gabrd*^{-/-} mice [73]. These data suggest that both synaptic and extrasynaptic GABA_ARs may be involved in the pathophysiology of depression.

Epilepsy

Alterations in numerous GABA_AR subunits have been documented in postmortem studies from epileptic patients, including alterations in extrasynaptic GABA_ARs (for review see [74]). An increase in the expression of the extrasynaptic GABA_AR $\alpha 5$ subunit, which mediates tonic GABAergic inhibition in CA1 pyramidal neurons [75], as well as an increase in GABA_AR δ subunit expression, which mediates tonic GABAergic inhibition in dentate gyrus granule cells (DGGCs) [76] have been documented in epileptic patients. These data are supported by the evidence that tonic GABAergic inhibition is preserved in tissue from epileptic patients [77] and demonstrate a role for extrasynaptic GABA_ARs in epilepsy.

These data are consistent with alterations observed in animal models of epilepsy. The breadth of changes, some conflicting and subregional or model specific, described in animal models is too cumbersome to detail in a short review. However, many reviews have covered this prolific topic [74]. Therefore, this review will focus solely on changes in extrasynaptic GABA_ARs, the best documented of which is a profound decrease in the GABA_AR δ subunit [78,79]. Despite the decreased expression of the GABA_AR δ subunit, which mediates tonic GABAergic inhibition in DGGCs, there is an increase in tonic GABA-ergic inhibition [80],

which may be because of a compensatory upregulation of the extrasynaptic GABA_AR $\alpha 5$ subunit in DGGCs [81]. Consistent with a role of extra-synaptic GABA_ARs in epilepsy, mice deficient in the GABA_AR δ subunit exhibit increased seizure susceptibility [43] and mice with deficits in the GABA_AR $\alpha 5$ subunit exhibit hyperexcitability [82].

Shifting of the localization of subunits has also been demonstrated in animal models of epilepsy. In addition to a general decrease in the GABA_AR δ subunit, a more specific decrease in δ subunit labeling at perisynaptic locations on dentate granule cells was detected; however, tonic inhibition was maintained [79] in agreement with previous studies. This study also showed increased $\gamma 2$ subunit labeling at perisynaptic locations, which was paralleled by a corresponding decrease in the phasic inhibition of granule cells [79]. Thus in seizure models, changes in the subcellular location of GABA_AR subunits could limit the ability of the inhibitory system to respond to excessive excitation.

Stroke

Enhancing GABAergic inhibition following stroke has been proposed to be a logical pharmacological intervention [83], such that increasing GABAergic inhibition would seemingly provide protection against ischemia-associated excitotoxic cell death. Interestingly, however, it has been shown that benzodiazepines worsen functional recovery following stroke [84]. Deficits in GABA-ergic inhibition associated with stroke have been inferred from decreased benzodiazepine binding in stroke patients [85] and postmortem studies reveal a downregulation of the GABA_AR $\alpha 2$ subunit in the lesioned cortex associated with a stroke [86].

Animal models confirm the role of GABA_ARs associated with the pathophysiology of stroke, demonstrating a decrease in GABA_AR $\alpha 1$, $\alpha 2$, $\alpha 5$ and $\gamma 2$ and an increase in $\alpha 3$ subunit expression [87,88,89]. Elegant studies by Clarkson *et al.* implicate extrasynaptic GABA_ARs in the detrimental consequences of stroke, demonstrating that pharmacologically or genetically decreasing $\alpha 5$ or δ subunit-containing receptors increases functional recovery following stroke [90**].

Conclusions

Both synaptic and extrasynaptic GABA_ARs have clear implications for multiple disorders of the nervous system, and thus represent excellent targets for the development of novel therapeutics. However, little is known about the complex mechanisms that govern the specific localization and regulation of extrasynaptic GABA_ARs in particular. Future research is needed to clarify ways in which we can specifically target the function of extrasynaptic GABA_ARs for relevance in disease conditions. It is apparent that alterations in the expression of extrasynaptic GABA_ARs contribute to the pathogenesis of neurodevelopmental disorders, schizophrenia, depression, epilepsy, and stroke. However, studies showing specific changes in the subcellular localization and electrophysiological properties of extrasynaptic receptors in many of these disease states are lacking, highlighting the need for detailed assessment of both phasic and tonic inhibition, and the expression and localization of their respective subunits in animal models of pathological conditions.

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Figure 1.

Mechanisms regulating the accumulation of synaptic/phasic and extrasynaptic/tonic GABA_ARs. The basal GABA_A current represents a combination of phasic inhibitory currents in a background of extrasynaptic GABA_AR activation. Phasic GABA_AR currents (mIPSCs, left panel) arise from the release of GABA-containing vesicles from a presynaptic terminal directly opposed to receptors clustered at the corresponding postsynaptic specialization. In the case of tonic inhibition (right panel), low ambient concentrations of GABA (or other agonists such as taurine), result in tonic activation of high affinity extrasynaptic receptors. The tonic current can be measured by application of a specific GABA_A antagonist, which blocks both phasic and tonic channel activity, resulting in a shift of the holding current that can be interpreted as the tonic GABA_AR contribution. (Currents shown are schematics.) Both phasic and tonic forms of GABA_AR inhibition rely on the appropriate targeting/clustering and function of distinct classes of GABA_ARs containing α1–3, βx, γ2 and α4–6, βx, δ receptor subunits, respectively. Studies from multiple laboratories have begun to define the mechanisms regulating the accumulation of synaptic GABA_ARs. The scaffolding protein gephyrin has been indicated as a key player in localizing synaptic GABA_ARs. In the cytosol, gephyrin exists as a trimer and relies on cooperative interactions with collybistin and neuroligin2 for enrichment at the plasma membrane. The complex of gephyrin–collybistin–neuroligin2, via interaction with presynaptic neurexins, helps to align the postsynaptic specialization with presynaptic GABA-releasing terminals. The clustering of GABA_ARs in the postsynaptic specialization is facilitated by interaction of specific subunits (α1, α2, α3) with gephyrin. In contrast to synaptic receptors, the proteins interacting with, and regulating the clustering of extrasynaptic receptors are largely unknown. To this point, only radixin has been indicated to interact with the α5 subunit, which is known to be present at extrasynaptic sites.