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$GABA_A$ Receptor Heterogeneity at the Synapse: the α-Subunit Subtype Story

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Abstract
$GABA_A$ receptors mediate the majority of fast inhibitory neurotransmission in the adult CNS. Given its widespread role, it is not surprising that $GABA_A$ receptors have diverse subunit compositions and receptor properties to facilitate inhibitory neurotransmission in disparate neuronal networks. The functional heterogeneity of $GABA_A$ receptors is strongly dependent on the receptor's subunit composition. Of particular interest for this review are the synaptic $GABA_A$ receptor α-subunit subtypes of the adult CNS which display the highest degree of heterogeneity relative to other subunit families. Each α-subunit subtype confers unique biophysical properties and distinct temporal expression patterns to their respective receptor isoform. This review will explore how these factors contribute to $GABA_A$ receptor heterogeneity which has great implications for both $GABA_A$ receptor function, as well as, pathologies that result from impaired $GABA_A$ mediated neurotransmission, such as age-dependent epilepsy.

Key Words: *receptor heterogeneity; synaptic GABAA receptors; phasic inhibition; α-subunit subtype; epilepsy*

Introduction

GABA-A receptors ($GABA_A$Rs) are ligand-gated ion channels that mediate fast inhibitory neurotransmission in the adult central nervous system (CNS)[1]. They belong to the gene family of Cys-loop, ligand-gated ion channels (LGIC) which include other receptors such as the nicotinic acetylcholine (nAChRs) and, the glycine receptors (GlyRs)[2,3]. Similar to most members of this family, $GABA_A$ receptors are heteropentamers assembled from a large array of homologous subunits such that, when viewed top-down from the synaptic cleft (Figure 1a), the receptors are predicted to have a circular structure with individual subunits arranged pseudosymmetrically around a central ion-conducting pore (Figure 1a)[2,3]. Thus far, nineteen (19) individual subunit subtypes, grouped according to sequence homology into eight (8) subunit families, have been identified (α1-6, β1-3, γ1-3, δ, ε, θ, π, ρ1-3)[4]. Each subunit subtype imparts unique biophysiological characteristics to their respective $GABA_A$ receptor isoforms[5-7], and exhibits distinct patterns of temporal expression dominance[8]—characteristics that will be discussed in the body of the review. Such an extensive repertoire of $GABA_A$ receptor subunits alludes to the potential for both promiscuous subunit combinations and diverse $GABA_A$ receptor properties. However, despite the relatively enormous possibilities for receptor combinations, the most predominant $GABA_A$ receptor isoform is composed of two (2) α-subunits, two (2) β-subunits and one(1) γ- or δ-subunit (Figure 1a), with the majority of receptors comprised of the γ2-subunit subtype[1], as well as a single type of α-subunit[9,10]. Nonetheless, intrinsic properties imparted by either subunit, particularly those of the α-subunit family, adequately diversify the characteristics of this predominant receptor isoform, thereby increasing $GABA_A$ receptor heterogeneity and subsequent utility within the diverse neuronal networks of the CNS[11].

The Relationship Between $GABA_A$ Receptor Function and Ligand Binding

$GABA_A$ receptor signaling in the adult nervous system is mediated by γ-aminobutyric acid (GABA), the most abundant inhibitory neurotransmitter in the mature CNS. GABA exerts its fast inhibitory effects by interacting with the $GABA_A$ receptor at two ligand binding sites, each located between the α- and β- subunit[12,13] (Figure 1a). Upon ligand binding, the $GABA_A$ receptor undergoes conformational changes, resulting in a net entry of chloride ions through the channel's pore. This net entry of anions permits a hyperpolarizing postsynaptic potential thereby reducing the probability of generating an action potential[1,14,15].

All $GABA_A$ receptor subunits share a similar structure which include: a large, extracellular N-terminus with ligand binding sites, four (4) alpha-helical transmembrane domains (TM1-4), a large cytoplasmic loop (M3-M4 loop), and an extracellular M2-M3 linker[16] (Figure 1b). These structural features are important determinants of receptor function, particularly $GABA_A$ receptor gating which represents the receptor's transition between the closed, open (ion-conducting) and desensitized (long-lived, agonist bound closed) states. $GABA_A$ receptor gating is most often induced by ligand binding in the N-terminus which is presumed to cause conformational changes within this region. Furthermore, it is believed that the ligand binding-induced conformational changes in the N-terminus are subsequently transduced down the receptor to its transmembrane domains and linker regions; thereby resulting in channel opening and $GABA_A$ receptor mediated signaling. One structural feature that contributes to efficient $GABA_A$ receptor gating is the M2-M3 linker (Figure 1b), which is suspected to couple agonist binding in the N-terminus to conformational changes in the receptor's transmembrane domains[17,18]. This coupling is believed to result from

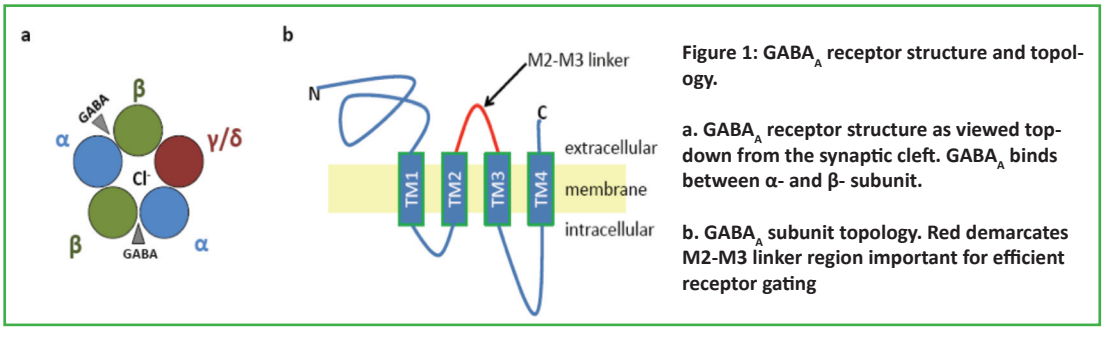

Figure 1: $GABA_A$ receptor structure and topology.

a. $GABA_A$ receptor structure as viewed top-down from the synaptic cleft. $GABA_A$ binds between α- and β- subunit.

b. $GABA_A$ subunit topology. Red demarcates M2-M3 linker region important for efficient receptor gating

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electrostatic interactions between aspartate residues within the N terminus and a highly conserved lysine residue in the M2-M3 linker region. Indeed, non-conserved amino acid changes of this lysine residue has been implicated in pathologies (e.g. epilepsy) that strongly suggest impairment of $GABA_A$ receptor gating[19-21]. Though the structural features of the receptor facilitates its cardinal properties, additional factors such as the receptor's intrinsic qualities imparted by diverse subunit compositions as well as the temporal profile of the receptor's exposure to GABA further determines the nature of $GABA_A$ mediated signaling[15].

$GABA_A$ Receptors Demonstrate Receptor Heterogeneity Through Two Types of Inhibitory Neurotransmission

As discussed in the previous paragraph, the nature of $GABA_A$ receptor neurotransmission may be influenced by both diverse receptor subunit composition and the temporal profile of receptor's exposure to GABA. Such influences are demonstrated by the two modes of $GABA_A$ mediated neurotransmission: tonic and phasic inhibition[22]. Tonic inhibition describes the continuous activation of extrasynaptic $GABA_A$ receptors by low concentrations (~1μM)[23] of ambient GABA owing to spill-over from the synaptic cleft. Given the environment within which they function, i.e. relatively far from the site of active GABA release[24], it is not surprising that these receptors have befitting biophysical properties, namely: a higher sensitivity to GABA and a significantly slower rate of receptor desensitization[A] compared to their synaptic counterparts[7,25,26]. These highly efficacious properties are largely bestowed by the δ-subunit, that with the exception of α5βxγ2 receptors, is the predominant subunit within extrasynaptic receptors[7,27,28] (Figure 1a). Furthermore, these predominant δ-subunit containing receptors are also largely comprised of the α4- or α6-subunit that additionally enhance the heterogeneity of extrasynaptic receptors in the CNS[8,26,27,29,30]. Though $GABA_A$ receptor- mediated tonic inhibition is an important component of inhibitory tone in the brain, henceforth, this review will focus on the other type of $GABA_A$ mediated neurotransmission: phasic inhibition.

Phasic Inhibitory Neurotransmission and Synaptic $GABA_A$ Receptor Function

In contrast to tonic inhibition, phasic inhibition refers to the transient activation of synaptic $GABA_A$ receptors by saturating concentrations (~1mM)[31] of GABA released from presynaptic terminals[32,33]. Consequent to GABA release, the synaptic $GABA_A$ receptors experience a rapid but short exposure to GABA that results from the ligand's rapid diffusion away from its release site[34]. This defining feature of the phasic mode of receptor activation gives rise to inhibitory postsynaptic currents (IPSCs) that activate rapidly and desensitize extensively[25] resulting in transient IPSCs. Therefore, unlike tonic inhibitory neurotransmission, phasic synaptic communication is tailored to facilitate the rapid and precise transmission of presynaptic activity into post synaptic signals.

Synaptic $GABA_A$ receptors also share a predominant subunit, namely the γ2-subunit, which confers unique biophysical properties ideally suited to mediate rapid, inhibitory neurotransmission[1]. Accordingly, γ2-containing synaptic receptors have faster activation and desensitization rates as compared to δ-containing receptors. Besides this function, γ2-subunits also play a central role in localizing these receptors at GABAergic synapses through interactions with the microtubule-associated protein, gephyrin[35,36]. Though the precise molecular interactions between the γ2-subunit and gephryin are yet to be established[37], *in vivo* experiments strongly demonstrate that gephryin plays a central role in clustering synaptic GABAA receptors[38]. In addition to gephryin stabilization of synaptic GABAA receptors, heterogeneity of receptor localization within post-synaptic densities is further increased by the receptor's α-subunit composition.

Synaptic α-Subunits and Heterogeneity of $GABA_A$ Receptor Localization

Subcellular localization of synaptic $GABA_A$ receptors is further enhanced by the α-subunit subtypes incorporated into the γ2-containing receptors. Synaptic receptors are predominantly composed of α1, α2, or α3 subunits[39,40] (Figure 1a), with the majority (~60%) of synaptic receptors comprised of the α1 subunit—a theme that will be discussed later in the review. Within the postsynaptic specialization of GABAergic synapses, α1-containing receptors are the most predominant, while the α2-containing receptors are particularly enriched within the axon initial segment (AIS) of neurons in the mature CNS[24] . In addition to distinct subcellular localizations, these α-subunit subtypes also differentially influence the biophysical properties of their respective synaptic receptors.

α-Subunit Subtypes Increase the Functional Heterogeneity of Synaptic $GABA_A$ Receptors

The heterogeneity of synaptic $GABA_A$ receptor's biophysical properties is enhanced by the α-subunit subtype composition of the receptor[5]. One biophysical property is the receptor's sensitivity to GABA, i.e., how effectively does GABA, once bound, promote $GABA_A$ receptor gating[41]. This property may be gauged by the receptor's EC50 which is a measure of the concentration of GABA that gives the half-maximal response such that receptors with a lower EC50 value have a higher sensitivity to the ligand and vice versa. For recombinant $GABA_A$ receptors, sensitivity to GABA is most strongly affected by the type of α-subunit subtype present, with α1-subunit containing synaptic receptors having the lowest EC50, the α2-containing receptors demonstrating an intermediate EC50 value and that of the α3-subunit subtype displaying the highest EC50 value[5,42,43].

Additional α-subunit-influenced biophysical properties include activation[B] and deactivation rates[C] . Regarding synaptic $GABA_A$ receptors' activation rates, experiments with recombinant receptors demonstrate the rank order to be α2<α1<α3 (Table 1), whereby α2-containing synaptic receptors have the fastest activation rate while α3 has the slowest[5,43,45]. On the other hand, deactivation rates are in the order α1<α2<α3[5,15,45] (Table 1), indicating that α2- and α3- containing receptors have prolonged currents relative to that of α1-containing receptors.

α-Subunit Subtypes Demonstrate Distinct Temporal Expression Patterns of Dominance

Remarkably, synaptic α-subunits increase GABAA receptor heterogeneity not only by contributing intrinsic biophysical properties but by also

[A] **Desensitization:** decrease in response amplitude in the continued presence of ligand.
[B] **Activation Rate:** time required for current onset to rise from 10%-90% of peak current.
[C] **Deactivation Rate**: tate of decrease in response amplitude after the removal of ligand application.

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influencing the temporal dominance of receptor isoform expression in the CNS. *In situ* hybridization and immunohisotchemical experiments in rodent models demonstrate that the most marked change in expression occurs for the α-subunit family that may result from the changing role of GABA during development[46]. As indicated previously in the review, the α1-subunit is the most predominantly expressed α-subunit in the adult CNS; however, it is minimally expressed in the developing brain. Conversely, the α2- and α3- subunits are predominantly expressed in the immature brain, with the α3-subunit demonstrating the higher expression of the two subunit subtypes[8,47]. Nevertheless, by P12 (in rodent models) the relative abundance of the developmentally predominant α2- and α3- subunits diminishes and there is a concomitant increase to dominancy in the α1-subunit expression in most brain regions[8]. Additional evidence for this age-dependent switch has also been demonstrated in nonhuman primate models[48], as well as human post mortem CNS tissue[49] where expression levels of the α-subunits mirror the age-dependent changes exhibited in rodent models, albeit with a more protracted trajectory.

These dynamic changes in the temporal dominance of α-subunit expression allude to a temporal change in composition as well as biophysical properties of $GABA_A$ receptors within the CNS. In fact, electrophysiological experiments in rodent models support this age-dependent change in dominant α-subunit receptor composition as early postnatal IPSCs display slow-deactivating currents relative to the faster deactivating IPSCs observed in the adult neurons (Table 1); indicative of a switch in predominant expression from α2-/α3- to α1-containing receptors[50-54]. Though the dominant α-subunit switching during development and the accompanied changes in GABAergic IPSCs properties are a testament to the dynamic role α-subunits play in increasing $GABA_A$ receptor heterogeneity, the functional relevance of this α-subunit-induced increased heterogeneity is not fully understood[51], particularly at the level of the neuronal network. However examining GABAergic pathologies, such as epilepsy, may assist in enumerating the role of α-subunit-induced $GABA_A$ receptor heterogeneity on neuronal network function.

Age Dependent Epilepsy: Implications of Dynamic α-Subunit Influenced $GABA_A$ Receptor Heterogeneity

Impairment of $GABA_A$ receptor function has been linked to the pathogenesis of idiopathic generalized epilepsy (IGEs)—a category of epilepsy syndromes believed to have a strong genetic underpinning[20,21,55]. Epilepsy affects approximately 0.5-1% of the general population and is defined as recurrent, unprovoked seizures that may result from lowered inhibition (disinhibition) of neuronal networks[56]; a possible consequence of $GABA_A$ receptor dysfunction. Specifically, seizures are threshold events; any point below an individual's seizure threshold can transform that individual into a seizure prone state[57]. Therefore, small changes in $GABA_A$ receptor mediated signaling, such as those induced by genetic mutations, may reduce an individual's seizure threshold and increase their probability of seizure onset. $GABA_A$ receptor mutations, such as those within the N terminus (R43Q) and the M2-M3 linker region (K289M) of the γ2-subunit (Figure 1), have been implicated in IGE pathology[19,58]. Both *in vitro* and *in vivo* experiments strongly suggest a mutation-induced disinhibition as a mechanism for epileptogenesis[20,21,59]. Furthermore, individuals with either mutation (R43Q and K289M) carry a number of phenotypes, one of which is febrile seizures (FSs). FSs are convulsions occurring during a febrile illness with an onset of six (6) months of age. However, the seizure occurrence spontaneously remit at six (6) years of age[60] and, interestingly, the individual's risk of developing epilepsy later in life (> 6 years) is only slightly greater than that of the general population[61].

The mechanism underlying this age-dependent, spontaneous remission is unknown but one hypothesis enumerates the dynamic changes in the temporal expression of $GABA_A$ receptor subunits as a possible agency for the changes in both seizure threshold and consequent seizure susceptibility[62]. As discussed in this review, members of the α- subunit family demonstrate the most dynamic temporal changes and confer distinct properties to their respective receptor isoform. Quite possibly, different α-subunit compositions may confer disparate properties to mutation containing receptors, such as those comprised of either γ2-subunit mutations discussed above. Therefore, a progressive change in $GABA_A$ receptor α- subunit composition and biophysical properties from that of α2-/α3- to that of α1- subunit subtypes may compensate for the mutation-induced receptor dysfunction; therefore providing a possible explanation for the age-dependent remission of epilepsy.

Table 1: Properties of α1-, α2-, α3- subunits containing GABAA receptors

α_x-subunit subtype	Subcellular Localization[24]	EC50 of αxβγ2 in response to GABA[5,43,45]	Rate of αxβγ2 Activation by GABA[5,45]	Rate of αxβγ2 Deactivation[5,45]	Expression levels of αxβγ2 in adult CNS[1]	Period of Predominance in CNS[45,47]
α1	Soma/dendrites	Low	Intermediate	Fast	60%	Adult/Mature
α2	AIS[1]	Intermediate	Fast	Intermediate	15-20%	Immature
α3	---	High	Slow	Slow	10-15%	Immature

[1]AIS: Axon Initial Segment; ---: no predominant area of localization

Conclusion

$GABA_A$ receptors play a major role in mediating inhibitory transmission in the CNS, which if perturbed, can contribute to pathologies such as epilepsy. Given its widespread function, it is not surprising that the properties of synaptic $GABA_A$ receptors and its subsequent inhibitory tone demonstrate heterogeneity. As discussed, the heterogeneity of $GABA_A$ receptors is increased by its subunit composition. Particularly, the α-subunit composition of the receptor influences the heterogeneity of GABAergic synapses. The relevance of this receptor heterogeneity, however, has not yet been fully elucidated. Nonetheless, pathologies such as age-dependent epilepsy, which implicate impaired $GABA_A$ mediated signaling, may offer further understating of the impact of α-subunit-induced $GABA_A$ receptor heterogeneity on inhibitory neurotransmission within neuronal networks.

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