The Functional Role of Spontaneously Opening GABA\textsubscript{A} Receptors in Neural Transmission

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Ionotropic type of $\gamma$-aminobutyric acid receptors (GABA\textsubscript{A}Rs) produce two forms of inhibitory signaling: phasic inhibition generated by rapid efflux of neurotransmitter GABA into the synaptic cleft with subsequent binding to GABA\textsubscript{A}Rs, and tonic inhibition generated by persistent activation of extrasynaptic and/or perisynaptic GABA\textsubscript{A}Rs by GABA continuously present in the extracellular space. It is widely accepted that phasic and tonic GABAergic inhibition is mediated by receptor groups of distinct subunit composition and modulated by different cytoplasmic mechanisms. Recently, however, it has been demonstrated that spontaneously opening GABA\textsubscript{A}Rs (s-GABA\textsubscript{A}Rs), which do not need GABA binding to enter an active state, make a significant input into tonic inhibitory signaling. Due to GABA-independent action mode, s-GABA\textsubscript{A}Rs promise new safer options for therapy of neural disorders (such as epilepsy) devoid of side effects connected to abnormal fluctuations of GABA concentration in the brain. However, despite the potentially important role of s-GABA\textsubscript{A}Rs in neural signaling, they still remain out of focus of neuroscience studies, to a large extent due to technical difficulties in their experimental research. Here, we summarize present data on s-GABA\textsubscript{A}Rs functional properties and experimental approaches that allow isolation of s-GABA\textsubscript{A}Rs effects from those of conventional (GABA-dependent) GABA\textsubscript{A}Rs.

Keywords: GABA-A receptor, GABA-independent inhibition, phasic conductance, tonic conductance, G-proteins

INTRODUCTION

Ionotropic receptors of $\gamma$-aminobutyric acid (GABA receptors of type A, GABA\textsubscript{A}Rs) are the main receptor type that generates inhibitory interneuronal signaling in the brain. The classical form of GABA\textsubscript{A}R-induced inhibitory signal is phasic inhibition: a short synchronized opening of GABA\textsubscript{A}Rs in a synapse, generated by the binding of GABA released from a presynaptic terminal. However, there is an alternative form of inhibition: charge transfer through continuously active GABA\textsubscript{A}Rs, or tonic inhibition, detected in peripheral nervous system in the 1970s (Brown, 1979) but documented for the central nervous system only in the 1990s (Otis et al., 1991; Brickley et al., 1996). The classical view is that tonic inhibition is generated in response to GABA, which is continuously present in the extracellular space of neural tissue due to spillover from synapses or release from astroglia and/or neurogliaform cells (Farrant and Nusser, 2005; Kozlov et al., 2006; Oláh et al., 2009). This implies the generation of a continuous inhibitory tone mainly by perisynaptic and extrasynaptic GABA\textsubscript{A}Rs, since the vast majority of transporters which perform reverse uptake of GABA are localized in synapses or in their immediate vicinity (Minelli et al., 1996; Chiu et al., 2002; Conti et al., 2004). Hence, the magnitude of tonic GABA\textsubscript{A}Rs-delivered current is considered to be regulated by the
availability of extracellular GABA, and by the quantity of GABA\_A\_Rs at an extrasynaptic surface of a given neuron (Glykys and Mody, 2007). Later research, however, revealed that a significant part of tonic inhibition mediated by GABA\_A\_Rs is independent of GABA binding, i.e., it is delivered by spontaneously opening GABA\_A\_Rs (s-GABA\_A\_Rs). s-GABA\_A\_Rs in that study were shown to be insensitive to the competitive GABA antagonist SR-95531 (SR), but could be suppressed by the GABA\_A\_R open channel blocker picrotoxin (PTX), and, to the less extent, by competitive GABA antagonist bicuculline (BIC; McCartney et al., 2007).

In the last few decades, studies of GABA\_A\_Rs-mediated tonic currents have attracted a considerable interest, and have described a functional role of this form of inhibition in a number of brain areas; in particular, its important input into neural excitability, synaptic plasticity, neurogenesis and network oscillations (Mody and Pearce, 2004; Farrant and Nusser, 2005; Glykys and Mody, 2007). Since our understanding of underlying mechanisms is still far from excellent, the newly discovered type of tonic conductance delivered via s-GABA\_A\_Rs promises a conceptual breakthrough in the field. Nevertheless, despite the phenomenon of GABA-independent gating of GABA\_A\_Rs being reported in numerous publications (Neelands et al., 1999; Birnir et al., 2000; Maksay et al., 2003; Miko et al., 2004), until recently the functional role of s-GABA\_A\_Rs in living neural tissue has remained beyond the focus of neuroscience research.

In this article, we try to summarize the data available to date on s-GABA\_A\_Rs function in neural transmission and to discuss perspective directions for further studies which should clarify the role of s-GABA\_A\_Rs under normal conditions and in pathology.

**FUNCTIONAL PROPERTIES OF s-GABARs**

**s-GABARs: Problem of the Isolation of GABA-Independent Effects**

One of the main factors which prevent a detailed study of s-GABA\_A\_Rs functioning is a lack of specific pharmacological tools: the independence of s-GABA\_A\_Rs gating from GABA binding makes impossible the use of competitive GABA antagonists for selective s-GABA\_A\_Rs silencing, whereas allosteric modulators such as benzodiazepines display a lack of specificity, tuning both GABA-dependent and GABA-independent effects (Bianchi and Macdonald, 2001; McCartney et al., 2007; Gerak, 2009).

Hence, to clarify the input of s-GABA\_A\_Rs into a given effect, differences in molecular mechanisms of SR- and PTX-induced GABA\_A\_Rs silencing have been used. SR is a competitive antagonist and thus negates GABA\_A\_R activity induced by GABA binding (i.e., it acts on conventional GABA\_A\_Rs); in contrast, PTX binds inside the GABA\_A\_R ion channel, and thus blocks all open channels, independently of the presence of GABA binding (i.e., it acts on both conventional GABA\_A\_Rs and s-GABA\_A\_Rs). Therefore, conventional GABA\_A\_R activity can be assessed as the change in the given effect obtained in the control vs. after application of SR, whereas s-GABA\_A\_R activity can be measured as the change in the effect obtained after SR application vs. after subsequent application of SR+PTX (Wlodarczyk et al., 2013)—see Figure 1. SR is a “silent” competitor for the GABA-binding site, i.e., it does not display inverse agonist properties. Obviously, competitive antagonists such as BIC, which display inverse agonism, cannot be used for the quantitative assessment of s-GABA\_A\_Rs effects: BIC was shown not only to suppress synaptic events as SR does but also to induce an outward shift of holding current (Wlodarczyk et al., 2013).

**s-GABARs Single-Channel Properties**

The obvious step in the biophysical characterization of different subgroups of ionotropic receptors is a dissection of single-channel properties, such as electrical conductance, opening frequency and average open time. Single-channel recordings have repeatedly demonstrated similar or very close conductance values for s-GABA\_A\_Rs and conventional GABA\_A\_Rs (Mathers, 1985; Neelands et al., 1999; Birnir et al., 2000; McCartney et al., 2007).
O’Neill and Sylantyev, 2018a,b) thus making this parameter hardly applicable for distinguishing between two receptor subtypes. Similarly, the dependence of GABA<sub>R</sub>Rs opening frequency on the concentration of GABA, makes this parameter inapplicable for discrimination of effects of s-GABA<sub>R</sub>Rs and conventional GABA<sub>R</sub>Rs in single-channel recordings. In contrast, the average open time was found to be significantly lower for s-GABA<sub>R</sub>Rs than for conventional GABA<sub>R</sub>Rs. This generates a two-peak distribution of opening time values under physiological conditions when free GABA is present in extracellular space (O’Neill and Sylantyev, 2018a). Earlier observations demonstrated that the two-peak Gaussian distribution of average open times is a characteristic feature of GABA<sub>R</sub>Rs of at least three different subunit compositions (Mortensen et al., 2010). It is important to note that the mode values for shorter durations in that work were found to be similar, irrespective of the agonist’s type and concentration, values for shorter durations in that work were found to be similar, irrespective of the agonist’s type and concentration, making this parameter thus applicable for distinguishing between two receptor subtypes. Similarly, the dependence of GABA<sub>R</sub>Rs opening frequency on the concentration of GABA, makes this parameter inapplicable for discrimination of effects of s-GABA<sub>R</sub>Rs and conventional GABA<sub>R</sub>Rs in single-channel recordings. In contrast, the average open time was found to be significantly lower for s-GABA<sub>R</sub>Rs than for conventional GABA<sub>R</sub>Rs. This generates a two-peak distribution of opening time values under physiological conditions when free GABA is present in extracellular space (O’Neill and Sylantyev, 2018a). Earlier observations demonstrated that the two-peak Gaussian distribution of average open times is a characteristic feature of GABA<sub>R</sub>Rs of at least three different subunit compositions (Mortensen et al., 2010). It is important to note that the mode values for shorter durations in that work were found to be similar, irrespective of the agonist’s type and concentration, thus representing an agonist-independent input. This suggests that: (i) s-GABA<sub>R</sub>Rs activity is a common element of integral GABA<sub>R</sub> response; and (ii) that s-GABA<sub>R</sub>Rs represent a functionally similar receptor subgroup composed of receptors of various subunit compositions.

Another method of distinguishing between s-GABA<sub>R</sub>Rs and conventional GABA<sub>R</sub>Rs at a level of single-channel effects may potentially develop from the recent observation about the ability of benzodiazepine flurazepam to modulate GABA-dependent and GABA-independent GABA<sub>R</sub>Rs function delivered via different molecular mechanisms (Jatczak-Śliwa et al., 2018).

s-GABA<sub>R</sub>Rs Input Into Tonic Conductance

Overall, charge transfer with phasic events mediated by GABA<sub>R</sub>Rs (and induced by GABA binding) compared to that delivered by tonic conductance through GABA<sub>R</sub>Rs, displays a ratio of more than 9/1 (Cope et al., 2005; O’Neill and Sylantyev, 2018a). Taking into account that GABA-induced tonic current was found to be negligible under physiological concentrations of extracellular GABA, whereas under these conditions s-GABA<sub>R</sub>Rs generated a significant amount of tonic current (Wlodarczyk et al., 2013), s-GABA<sub>R</sub>Rs should be considered as a potential key element in the generation of lasting inhibitory tone and, in a wider context, in inter-neuronal crosstalk.

Tonic inhibition has been widely accepted to be a strong modulator of action potential (AP) generation (Hamann et al., 2002; Bonin et al., 2007), AP firing patterns (Häußer and Clark, 1997) and the coincidence detection time window for synaptic inputs (Tang et al., 2011). Experiments on s-GABA<sub>R</sub>Rs have readily confirmed their significant input into the regulation of the following phenomena: the modulation of AP generation (O’Neill and Sylantyev, 2018b), firing patterns (Botta et al., 2015; O’Neill and Sylantyev, 2018a), neurons’ rheobase, and the time window of coincidence detection of excitatory inputs (O’Neill and Sylantyev, 2018a).

s-GABA<sub>A</sub>Rs Input Into Phasic Conductance

Several classical studies have demonstrated that GABA<sub>A</sub>Rs of specific subunit compositions (e.g., δ-GABA<sub>A</sub>Rs) which may be responsible for a lion’s share of tonic current (Nusser and Mody, 2002; Stell et al., 2003; Mortensen et al., 2010) are localized exclusively at the extrasynaptic membrane (Nusser et al., 1998; Wei et al., 2003). However, if s-GABA<sub>A</sub>Rs are a functionally similar group of receptors of different subunit composition (see “s-GABA<sub>R</sub>Rs Single-Channel Properties” section), their absence in synapses would be highly doubtful. This, in turn, raises a question as to how (and whether) s-GABA<sub>A</sub>Rs modify synaptic (phasic) GABA-ergic inhibitory responses (inhibitory post-synaptic currents, IPSCs). In truth, recent studies have demonstrated their significant input into IPSC decay kinetics: s-GABA<sub>A</sub>Rs introduced a slow element of decay profile (O’Neill and Sylantyev, 2018a), probably due to their higher potency to GABA (Yeung et al., 2003) and/or modified receptor efficacy.

It was shown earlier that GABA<sub>A</sub>R-generated IPSC may contain fast and slow components with different sensitivities to GABA competitive antagonists, which resembles the functional profile of s-GABA<sub>A</sub>Rs (Kapur et al., 1997). In this research, the generation of fast and slow components of whole-cell IPSC was attributed to different cell regions: dendritic and somatic, respectively. On the other hand, later direct recordings of s-GABA<sub>A</sub>Rs activity confirmed a significant input of this receptor subtype into both whole-cell IPSCs (which are generated in synapses), and into IPSCs evoked in nucleated membrane patches, i.e., generated by GABA<sub>A</sub>Rs localized at a neuronal cell soma (O’Neill and Sylantyev, 2018a). On top of that, a significant input of δ-GABA<sub>A</sub>Rs into IPSCs was recently demonstrated (Sun et al., 2018), which confirms once again both the synaptic and extrasynaptic localization of GABA<sub>A</sub>Rs which display high tonic activity.

Intracellular Regulatory Mechanisms of s-GABA<sub>A</sub>Rs Activity

The particular intracellular mechanisms which are used by neural cells to modulate the activity of GABA<sub>A</sub>Rs are still far from being completely understood; however, it has long been established that direct phosphorylation is of major importance (Brandon et al., 2002). It was shown that GABA<sub>A</sub>Rs functions can be modulated differentially (potentiated or suppressed) depending on the receptor subunit composition, the type of neuron, et cetera by cAMP-dependent protein kinase A (PKA), tyrosine kinase Src and PKC: refer to Brandon (2002) for review. In particular, GABA<sub>A</sub>R-mediated tonic inhibitory currents were shown to be downregulated by PKC Bright and Smart, 2013, whereas PKA was found to enhance this type of inhibition (Carlson et al., 2016). In addition, GABA<sub>A</sub>Rs effects were repeatedly shown to be modulated by G-protein-coupled receptors via G-proteins of different types (Cai et al., 2002; Wang et al., 2002) which are, in turn, tightly connected to the regulation of PKC and PKA activity (Neves et al., 2002). Hence, the clarification of impact on s-GABA<sub>A</sub>Rs function delivered by intracellular regulatory factors (specifically, by various kinases and G-proteins), is one of the key steps needed for understanding and predicting s-GABA<sub>A</sub>Rs functional input into a neural transmission.
To date, there is little data on this. It has been demonstrated that in dentate gyrus granule cells of hippocampus PKC regulates tonic GABA-dependent inhibitory conductance but has no significant impact on the GABA-independent effects of s-GABA<sub>R</sub>Rs (O’Neill and Sylantyev, 2018b). However, at a longer time scale it was repeatedly shown that PKC and Ca<sup>2+</sup>/calmodulin-dependent protein kinase II increase tonic inhibition in hippocampus and amygdala due to enhanced phosphorylation and membrane insertion of β3-containing GABA<sub>R</sub>Rs (Saliba et al., 2012; Modgil et al., 2017) and α4-containing GABA<sub>R</sub>Rs; this PKC action can be potentiated by neurosteroids such as THDOC (Abramian et al., 2010, 2014; Romo-Parra et al., 2015). In turn, s-GABA<sub>R</sub>Rs-mediated tonic inhibition in dentate gyrus granule cells is controlled by G-proteins: non-specific block of G-proteins by pertussis toxin decreases the tonic current via the reduction of the s-GABA<sub>R</sub> opening frequency (O’Neill and Sylantyev, 2018b).

In contrast to PKC, activation of PKA was found to increase the tonic current through αβ3δ and, to a lesser extent, αβ3γ2L-GABA<sub>R</sub>Rs in absence of GABA due to upregulation of single-channel opening frequency. Addition of GABA to an ambient solution, however, gradually decreased the sensitivity of GABA<sub>R</sub>Rs of both subunit compositions to modulation by PKA; such a modulation became insignificant when GABA concentration reached micromolar values (Tang et al., 2010). It is important to note, however, that a significant part of GABA-independent s-GABA<sub>R</sub>Rs activity was found to be out of the control of any soluble cytoplasmic factors. GABA-independent openings of GABA<sub>R</sub>Rs were recorded from outside-out patches excised from dentate gyrus granule cells somata: in this preparation, all cytoplasmic signaling chains are surely destroyed (O’Neill and Sylantyev, 2018b). However, anchored kinases that modulate ionotropic receptors (Brandon et al., 2003; Carnegie and Scott, 2003) may still be responsible for at least a part of the s-GABA<sub>R</sub> activity observed in outside-out patches.

**CONCLUSIONS AND FURTHER RESEARCH DIRECTIONS**

To date, there have been only a few publications highlighting the functional properties of s-GABA<sub>R</sub>Rs in living neurons. This imposes obvious limitations on conclusions in terms of the applicability for different brain regions and types of neurons. Nevertheless, the significant input of s-GABA<sub>R</sub>Rs into neural signaling varies widely, depending on the particular brain region and cell type. To the best of our knowledge, previous articles that discuss lower EC<sub>50</sub> values (i.e., higher potency) of extrasynaptic GABA<sub>R</sub>Rs in vivo do not consider spontaneous channels and how they influence such measurements. This fact enforces the importance of the work on s-GABA<sub>R</sub>Rs pharmacology for an understanding of biophysical phenomena in living neurons.

The important question regarding s-GABA<sub>R</sub>Rs is whether or not these receptors represent a convergent group with similar functional properties, or if they share common receptor subunit(s). Numerous studies have attributed the majority (up to 75%) of GABA<sub>R</sub>-delivered tonic inhibition to δ-containing GABA<sub>R</sub>Rs (Stell et al., 2003), which are abundant at extrasynaptic membranes (Nusser et al., 1998) but have been also found in synapses where they make a significant input into phasic inhibition (Sun et al., 2018), and in perisynaptic loci (Wei et al., 2003). The remaining portion of tonic inhibition is, to a large extent but not fully, produced by receptors containing the α5-subunit (Farrant and Nusser, 2005). Furthermore, the agonist-independent GABA<sub>R</sub> openings were observed under similar conditions for receptors of three different subunit compositions (Mortensen et al., 2010). In addition, the observation that mutations in α1 and β2 subunits modulate spontaneous GABA<sub>R</sub> gating (Baptista-Hon et al., 2017) prevents us from ruling out these subunits as potential alternative candidates to be involved in the formation of s-GABA<sub>R</sub>Rs. Combined with the facts of the GABA-independent tonic activity of α4-GABA<sub>R</sub>Rs (Tang et al., 2010) and spontaneous openings of α2β1γ-R-delivered tonic inhibition to δ-containing GABA<sub>R</sub>Rs which contribute to the baseline currents in whole-cell recordings (Wagner et al., 2005), the abovementioned data on GABA-independent activity suggest that GABA-independent inhibition is of poly-subtype origin, with a substantial part inherent in the non-δ- and non-α5-containing receptors.

In view of numerous subunits and subunit compositions of GABA<sub>R</sub>Rs which demonstrate spontaneous gating, the obvious question is: are there GABA<sub>R</sub> subtype(s) which do not demonstrate GABA-independent activity? The existence of such GABA<sub>R</sub>Rs was suggested by the study showing that, in contrast to the α2β1γ receptor, responses of α2β1γ2-GABA<sub>R</sub>Rs do not produce a “baseline overshoot” associated with spontaneous openings (Wagner et al., 2005).
Therefore, data collected to date suggest revision of two traditional views, now common in fundamental neuroscience: (i) that tonic inhibitory conductance is generated by ambient GABA (due to proven significance of s-GABA\textsubscript{Rs} input); and (ii) that tonic and phasic inhibition are mediated by different GABA\textsubscript{Rs} subtypes (due to growing evidence that typical extrasynaptic GABA\textsubscript{Rs} can make a significant contribution into IPSCs via a synaptic and/or perisynaptic presence).

It has been demonstrated that a scarcity of \alpha\textsubscript{1} subunit is correlated with resistance to anti-epileptic drugs (Bethmann et al., 2008), whereas increased \alpha\textsubscript{1}-GABA\textsubscript{AR} expression in the hippocampus suppresses the development of temporal lobe epilepsy (TLE; Raol et al., 2006). Apart from that, it was shown that phasic GABA-ergic inhibition is lowered in TLE, whereas tonic GABA-ergic conductance remains intact (Palma et al., 2007; Pavlov et al., 2011), making tonic GABA-ergic current a perspective target for TLE treatment. The classical paradigm, where extracellular GABA triggers tonic GABA-ergic current, implies that the most effective therapeutic approach is to increase the concentration of GABA in the cerebrospinal fluid, and thus augment inhibitory conductance. However, this approach was repeatedly found to be ineffective (Cohen et al., 2002; Glykys et al., 2009) or even one that leads to various side effects. These side effects impose limitations on the clinical use of specific antiepileptic drugs that increase this approach was repeatedly found to be ineffective (Cohen et al., 2002; Glykys et al., 2009) or even one that leads to various side effects due to growing evidence that typical extrasynaptic GABA\textsubscript{Rs} can make a significant contribution into IPSCs via a synaptic and/or perisynaptic presence.

Another clinical implication of s-GABA\textsubscript{Rs} rises from the fact that sedative and analgesic effects of gaboxadol (THIP) are mediated exclusively by \alpha\textsubscript{4}-containing GABA\textsubscript{Rs} (Chandra et al., 2006), that demonstrate GABA-independent activity.

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**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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