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The Functional Role of Spontaneously Opening GABA_A Receptors in Neural Transmission

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Ionotropic type of γ -aminobutyric acid receptors (GABA_ARs) produce two forms of inhibitory signaling: phasic inhibition generated by rapid efflux of neurotransmitter GABA into the synaptic cleft with subsequent binding to GABA_ARs, and tonic inhibition generated by persistent activation of extrasynaptic and/or perisynaptic GABA_ARs 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_ARs (s-GABA_ARs), 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_ARs 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_ARs 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_ARs functional properties and experimental approaches that allow isolation of s-GABA_ARs effects from those of conventional (GABA-dependent) GABA_ARs.

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

INTRODUCTION

Ionotropic receptors of γ -aminobutyric acid (GABA receptors of type A, GABA_ARs) are the main receptor type that generates inhibitory interneuronal signaling in the brain. The classical form of GABA_AR-induced inhibitory signal is phasic inhibition: a short synchronized opening of GABA_ARs 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_ARs, 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_ARs, 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_ARs-delivered current is considered to be regulated by the

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availability of extracellular GABA, and by the quantity of GABA_ARs 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_ARs is independent of GABA binding, i.e., it is delivered by spontaneously opening GABA_ARs (s-GABA_ARs). s-GABA_ARs in that study were shown to be insensitive to the competitive GABA antagonist SR-95531 (SR), but could be suppressed by the GABA_AR 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_ARs-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_ARs promises a conceptual breakthrough in the field. Nevertheless, despite the phenomenon of GABA-independent gating of GABA_ARs 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_ARs 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_ARs function in neural transmission and to discuss perspective directions for further studies which should clarify the role of s-GABA_ARs under normal conditions and in pathology.

FUNCTIONAL PROPERTIES OF s-GABARs

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

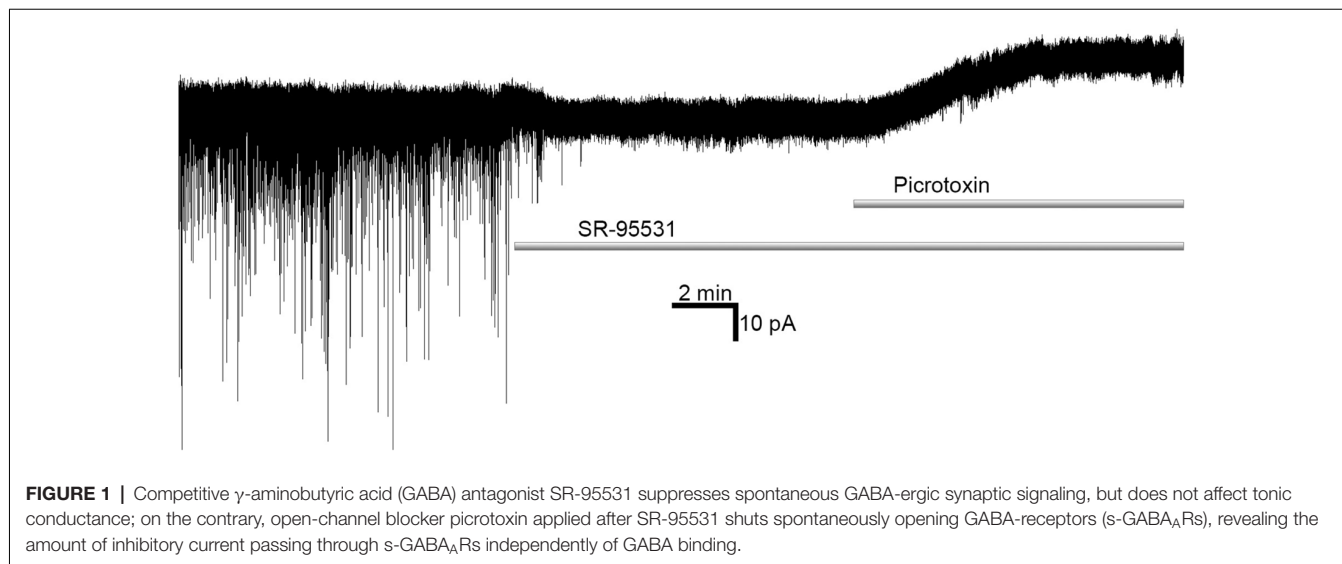
One of the main factors which prevent a detailed study of s-GABA_ARs functioning is a lack of specific pharmacological

tools: the independence of s-GABA_ARs gating from GABA binding makes impossible the use of competitive GABA antagonists for selective s-GABA_ARs 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_ARs into a given effect, differences in molecular mechanisms of SR- and PTX-induced GABA_ARs silencing have been used. SR is a competitive antagonist and thus negates GABA_AR activity induced by GABA binding (i.e., it acts on conventional GABA_ARs); in contrast, PTX binds inside the GABA_AR ion channel, and thus blocks all open channels, independently of the presence of GABA binding (i.e., it acts on both conventional GABA_ARs and s-GABA_ARs). Therefore, conventional GABA_AR activity can be assessed as the change in the given effect obtained in the control vs. after application of SR, whereas s-GABA_AR activity can be measured as the change in the effect obtained after SR application vs. after subsequent application of SR+PTX (Włodarczyk 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_ARs effects: BIC was shown not only to suppress synaptic events as SR does but also to induce an outward shift of holding current (Włodarczyk 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_ARs and conventional GABA_ARs (Mathers, 1985; Neelands et al., 1999; Birnir et al., 2000;



O'Neill and Sylantsev, 2018a,b) thus making this parameter hardly applicable for distinguishing between two receptor subtypes. Similarly, the dependence of GABA_ARs opening frequency on the concentration of GABA, makes this parameter inapplicable for discrimination of effects of s-GABA_ARs and conventional GABA_ARs in single-channel recordings. In contrast, the average open time was found to be significantly lower for s-GABA_ARs than for conventional GABA_ARs. This generates a two-peak distribution of opening time values under physiological conditions when free GABA is present in extracellular space (O'Neill and Sylantsev, 2018a). Earlier observations demonstrated that the two-peak Gaussian distribution of average open times is a characteristic feature of GABA_ARs 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_ARs activity is a common element of integral GABA_AR response; and (ii) that s-GABA_ARs represent a functionally similar receptor subgroup composed of receptors of various subunit compositions.

Another method of distinguishing between s-GABA_ARs and conventional GABA_ARs 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_AR gating *via* different molecular mechanisms (Jatczak-Śliwa et al., 2018).

s-GABARs Input Into Tonic Conductance

Overall, charge transfer with phasic events mediated by GABA_ARs (and induced by GABA binding) compared to that delivered by tonic conductance through GABA_ARs, displays a ratio of more than 9/1 (Cope et al., 2005; O'Neill and Sylantsev, 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_ARs generated a significant amount of tonic current (Włodarczyk et al., 2013), s-GABA_ARs 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_ARs have readily confirmed their significant input into the regulation of the following phenomena: the modulation of AP generation (O'Neill and Sylantsev, 2018b), firing patterns (Botta et al., 2015; O'Neill and Sylantsev, 2018a), neurons' rheobase, and the time window of coincidence detection of excitatory inputs (O'Neill and Sylantsev, 2018a).

s-GABA_ARs Input Into Phasic Conductance

Several classical studies have demonstrated that GABA_ARs of specific subunit compositions (e.g., δ -GABA_ARs) 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_ARs are a functionally similar group of receptors of different subunit composition (see "s-GABARs 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_ARs 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_ARs introduced a slow element of decay profile (O'Neill and Sylantsev, 2018a), probably due to their higher potency to GABA (Yeung et al., 2003) and/or modified receptor efficacy.

It was shown earlier that GABA_AR-generated IPSC may contain fast and slow components with different sensitivities to GABA competitive antagonists, which resembles the functional profile of s-GABA_ARs (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_ARs 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_ARs localized at a neural cell soma (O'Neill and Sylantsev, 2018a). On top of that, a significant input of δ -GABA_ARs into IPSCs was recently demonstrated (Sun et al., 2018), which confirms once again both the synaptic and extrasynaptic localization of GABA_ARs which display high tonic activity.

Intracellular Regulatory Mechanisms of s-GABA_ARs Activity

The particular intracellular mechanisms which are used by neural cells to modulate the activity of GABA_ARs 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_ARs 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 et al. (2002) for review. In particular, GABA_AR-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_ARs 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_ARs 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_ARs 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_ARs (O'Neill and Sylantsev, 2018b). However, at a longer time scale it was repeatedly shown that PKC and Ca²⁺/calmodulin-dependent protein kinase II increase tonic inhibition in hippocampus and amygdala due to enhanced phosphorylation and membrane insertion of β3-containing GABA_ARs (Saliba et al., 2012; Modgil et al., 2017) and α4-containing GABA_ARs; 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_ARs-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_ARs opening frequency (O'Neill and Sylantsev, 2018b).

In contrast to PKC, activation of PKA was found to increase the tonic current through α4β3δ and, to a lesser extent, α4β3γ2L-GABA_ARs 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_ARs 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_ARs activity was found to be out of the control of any soluble cytoplasmic factors. GABA-independent openings of GABA_ARs 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 Sylantsev, 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_ARs 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_ARs 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_ARs into the modulation of output signal generation and into the integration of input signaling in a given neuron, suggests that s-GABA_ARs activity is one of the key actors that regulate neural inhibition.

Indeed, the relative importance of GABA-independent s-GABA_ARs signaling in a given region of the brain depends critically on the native concentration of GABA in the extracellular space. Different groups report *in vivo* concentrations varying by more than an order of magnitude: from less than 100 (Włodarczyk et al., 2013) or 200 (Glaeser and Hare, 1975) nM to units of micromoles (Tossman et al., 1986; Takagi et al., 1993). Moreover, there may be local inhomogeneities of GABA concentrations due to cell-specific

differences in the distribution and/or activity of GABA transporters and the elements of the GABA synthesis system. This was indirectly confirmed by the observation that the silencing of GAD-65 activity reduces tonic inhibitory currents in interneurons, but not in the pyramidal neurons of the hippocampal CA1 area (Song et al., 2011). A recent study on the hippocampus has demonstrated that at a GABA concentration of ~100 nM, the amount of GABA-induced tonic current (which can be suppressed by SR) is close to statistical noise (see example at **Figure 1**), and negligible when compared to that through GABA-independent openings of s-GABA_ARs (Włodarczyk et al., 2013); on the contrary, SR has been shown to reveal a huge amount of tonic GABA-dependent current in thalamus (Cope et al., 2005). These data suggest that the relative impact of s-GABA_ARs 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 EC₅₀ values (i.e., higher potency) of extrasynaptic GABA_ARs *in vivo* do not consider spontaneous channels and how they influence such measurements. This fact enforces the importance of the work on s-GABA_ARs pharmacology for an understanding of biophysical phenomena in living neurons.

The important question regarding s-GABA_ARs 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_AR-delivered tonic inhibition to δ-containing GABA_ARs (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_AR 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_ARs 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_ARs. Combined with the facts of the GABA-independent tonic activity of α4-GABA_ARs (Tang et al., 2010) and spontaneous openings of α2β1ε-GABA_ARs 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_AR which demonstrate spontaneous gating, the obvious question is: are there GABA_ARs subtype(s) which do not demonstrate GABA-independent activity? The existence of such GABA_ARs was suggested by the study showing that, in contrast to the α2α1ε receptor, responses of α2β1 and α2β1γ2-GABA_ARs 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_ARs input); and (ii) that tonic and phasic inhibition are mediated by different GABA_ARs subtypes (due to growing evidence that typical extrasynaptic GABA_ARs can make a significant contribution into IPSCs *via* a synaptic and/or perisynaptic presence).

It has been demonstrated that a scarcity of $\alpha 1$ subunit is correlated with resistance to anti-epileptic drugs (Bethmann et al., 2008), whereas increased $\alpha 1$ -GABA_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 epileptogenesis (Palma et al., 2006; Cope et al., 2009) due to various side effects. These side effects impose limitations on the clinical use of specific antiepileptic drugs that increase the concentration of GABA in cerebrospinal fluid (Sander and Hart, 1990; Leppik, 1995). In contrast, the modulation of s-GABA_ARs in GABA-independent manner promises an alternative for TLE treatment through the regulation of tonic conductance without the need to interfere with extracellular

GABA concentration, thus avoiding the afore mentioned side effects.

Apart from the potential of $\alpha 1$ -GABA_ARs for TLE treatment, $\alpha 5$ -GABA_ARs (which also display GABA-independent activity) were found to be a perspective target for schizophrenia treatment (Lodge and Grace, 2011). Taking into account similar concentration of GABA found *in vivo* in the brains of schizophrenic patients and of a control group (Tayoshi et al., 2010), and the well-established fact that changes in tonic GABA-ergic inhibition are involved in the generation of schizophrenia symptoms (Damgaard et al., 2011), these data suggest a potentially important role of drugs targeting s-GABA_ARs in the suppression of schizophrenia development, since action through s-GABA_ARs in GABA-independent manner eliminates the need to modify GABA concentration in cerebrospinal fluid.

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

AUTHOR CONTRIBUTIONS

NO and SS contributed to the conception and design of the article. NO received data displayed at the figure and analyzed literature connected to the topic, contributed to manuscript revision. SS wrote the manuscript.

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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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