

# Distinguishing between GABA<sub>A</sub> Receptors Responsible for Tonic and Phasic Conductances\*

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Cell-to-cell communication in the mammalian nervous system does not solely involve direct synaptic transmission. There is considerable evidence for a type of communication between neurons through chemical means that lies somewhere between the rapid synaptic information transfer and the relatively non-specific neuroendocrine secretion. Here I review some of the experimental evidence accumulated for the GABA system indicating that GABA<sub>A</sub> receptor-gated Cl<sup>-</sup> channels localized at synapses differ significantly from those found extrasynaptically. These two types of GABA<sub>A</sub> receptor are involved in generating distinctly different conductances. Thus, the development and search for pharmacological agents specifically aimed at selectively altering synaptic and extrasynaptic GABA<sub>A</sub> conductances is within reach, and is expected to provide novel insights into the regulation of neuronal excitability.

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**KEY WORDS:** GABA; inhibition; neuronal excitability; GABA<sub>A</sub> receptors.

## INTRODUCTION

The intermediate type of neurotransmission, considered to lie between fast synaptic transmission and slow neuroendocrine secretion has been originally described for catecholamines and peptide neurotransmitters. It has been termed parasynaptic transmission (1), nonsynaptic diffusion neurotransmission (NDN) (2), volume transmission (3,4), or wireless transmission (5). In this review, the two types of transmission will be referred to as *phasic*, corresponding to the “wired transmission” (WT) and *tonic*, the equivalent of “volume transmission” (VT) (3,4). The VT type of interneuronal communication involves the diffusion of the neurotransmitter to sites nearby (0.1 μm to 100’s of μm) to

its release, and virtually every neurotransmitter system of the brain can operate in this mode (4–6).

About 15 years ago it became evident that mismatches between the location of release sites for certain neurotransmitters and receptors for the same transmitter are extremely abundant in the brain (7). Thus, experimental support was gained for the idea that such mismatches might represent high-affinity non-synaptic receptors underlying VT. Since then, it has become clear that merely by the spatial relationship between neurotransmitter receptors and synapses, neuronal signaling through most neurotransmitter systems has two distinct modes of operation: a synaptic component and another mediated by receptors found outside of the synaptic cleft. Such dual chemical intercellular communication may diminish the independence of individual synapses, and thus, by increasing the degrees of freedom for the possible modes of activation of the receptors, may actually lead to a reduction of the information processing power of the brain (8). However, the

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activation of extrasynaptic receptors may represent a background signal related to the overall level of neuronal activity present in a local network. Clearly, the differential modulation of the function and activation of these two signaling systems by various endogenous substances or by exogenously administered drugs (5,6) has significant consequences on the information processing functions of the brain during health and disease.

The differences between WT and VT for the GABA system have not been examined in detail, although it was clear from earlier recordings that a massive tonic inhibition is present in hippocampal neurons (9), and that it develops gradually in cerebellar granule cells to control neuronal excitability (10). The aim of the present review is to start addressing distinctions between phasic and tonic GABAergic mechanisms at morphological, molecular, pharmacological, and physiological levels. By understanding the distinguishing features of WT and VT at GABA<sub>A</sub> receptors we hope to gain insight into the general modulation of inhibition in the brain. This will have considerable implications for both normal physiological neuronal events (e.g., normal development, learning and memory, reproductive cycle) and pathological states of brain function (e.g., epilepsy, Alzheimer's, Huntington's or Parkinson's disease, and numerous psychiatric disorders including depression, panic disorders, anxiety, and schizophrenia) (11–13).

*Synaptic and Extrasynaptic GABA<sub>A</sub> Receptors.* Modulators of GABA<sub>A</sub> receptors are administered daily during surgical procedures as general anesthetics (14,15), and several widely used therapeutical approaches to the treatment of certain nervous system disorders rely on enhancing the effectiveness of GABAergic inhibition (16). Yet, the molecular diversity, physiology and topography of GABA<sub>A</sub> receptors at synapses and those found at extrasynaptic sites puts several major limitations on how the function of such receptors might be altered pharmacologically or physiologically. The high and transient GABA concentration in the synaptic cleft during synaptic transmission (17–19) and the varying degree of receptor occupancy at different synapses (20) should be an immediate concern for pharmacological experiments. Receptors will be differently modulated by drugs depending on the time-course and concentration of agonist (19). Moreover, the presence of specific extrasynaptic GABA<sub>A</sub> receptors (21,22) raises further questions about the unequivocal assessment of GABAergic pharmacological actions in the CNS. If GABA levels are between 0.5–1.0 μM in the extracellular fluid (23,24), extrasynaptic receptors may be continually activated by

ambient levels of GABA, albeit depending on their subunit composition they may be partially desensitized (17). There is a distinct control of cellular excitability by tonic and phasic GABAergic inhibition (10) and the two kinds of inhibition seem to have distinct pharmacological responses to both endogenous and exogenous compounds. The two types of inhibition may thus perform entirely different functions in the brain, and alterations in one or the other type of inhibition may have profound effects on the excitability of individual neurons and on the function of connected networks.

As pointed out, the differential activation of synaptic and extrasynaptic ionotropic receptors is by no means unique to the GABA system. Ambient levels of glutamate act on NMDA receptors found extrasynaptically (25,26) and significantly alter neuronal excitability. Thus, the distinction between WT and VT is a general property of amino acid neurotransmitter systems in the mammalian brain. The present review will exclusively address the activation of ionotropic GABA<sub>A</sub> receptors located at synaptic and extrasynaptic sites, while acknowledging that regulation of neuronal excitability through metabotropic GABA receptors (GABA<sub>B</sub>) is an equally important part of the tonic and phasic GABAergic actions in the brain (27).

*Specific Pharmacological Properties of Synaptic and Extrasynaptic GABA<sub>A</sub> Receptors.* The effects of drugs acting on central GABA<sub>A</sub> receptors have been mainly interpreted in the context of the essential role played by *synaptic* GABA<sub>A</sub> receptor-mediated inhibition in the brain. However, these drugs do not act only on *synaptic* GABA<sub>A</sub> receptors, but also change the properties of the receptors termed *extrasynaptic receptors* that are located outside the synaptic contacts. The number of these latter receptors may exceed that found at synapses (21) and, therefore they are in a position to exert significant effects on cellular and network excitability. Some studies have stressed the role of extrasynaptic GABA<sub>A</sub> receptors activated by low ambient concentrations of GABA, possibly “spilled over” following synaptic release (10,28,29). In cerebellar granule cells, the two distinct types of GABA<sub>A</sub> receptor-mediated inhibition have been termed phasic and tonic inhibition, respectively produced by synaptically released GABA acting on postsynaptic GABA<sub>A</sub> receptors, and by the persistent activation of extrasynaptic receptors by low concentrations of ambient GABA. Our present understanding is that the α4, α5, α6, and δ subunits may be the major candidates for GABA<sub>A</sub> receptor subunits with preferential extrasynaptic localization. These receptors assemble with other subunits to form functional receptors, and their

most likely natural combinations in the brain might be  $\alpha 4\beta \times \delta$ ,  $\alpha 5\beta 3\gamma 2/3$ ,  $\alpha 6\beta 2/3\delta$  (30). There might be other extrasynaptically located GABA<sub>A</sub> receptors, including the most abundant GABA<sub>A</sub> receptor type in the brain composed of  $\alpha 1\beta 2\gamma 2$  subunits (30). However, the regular association of the  $\gamma 2$  subunit with synaptic anchoring proteins like gephyrin (31) or GABARAP (32) makes this possibility somewhat less likely.

*The  $\alpha 6$  Subunit.* This subunit is found almost exclusively in cerebellar granule cells, and only minute amounts are present in a negligible number of other cell types (22). Pharmacological experiments in the cerebellum (29) have shown that the GABA<sub>A</sub> receptor subtypes underlying IPSCs elicited by an electrical stimulus are different than those activated following spontaneous transmitter release. Using furosemide as a specific blocker of the  $\alpha 6$  containing receptors (33) it was demonstrated that GABA release evoked by electrical stimulation, but not spontaneously released GABA, spills over to adjacent extrasynaptic receptors that comprise  $\alpha 6$  subunits. It was in these same granule cells where the initial distinction between tonic and phasic inhibition was made (10) with the  $\alpha 6$  subunits being the most likely candidates to be located extrasynaptically. Unfortunately the benzodiazepine (BZ) sensitivity of the receptors was not tested to provide insight into the presence of  $\gamma 2$  vs  $\delta$  subunits associated with the extrasynaptic  $\alpha 6$  subunits. Based on anatomical studies (see below), the  $\delta$  subunit is the most likely candidate to be associated with the  $\alpha 6$  subunits at extrasynaptic sites. Nevertheless, in a primary embryonic culture preparation, the tonic inhibition was reported to be flunitrazepam sensitive (34), indicating that instead of the BZ-insensitive  $\delta$  subunits, at least in cultured cerebellar granule cells,  $\gamma 2$  subunits can be present at extrasynaptic sites. This, however is not surprising in light of the findings that before the first postnatal week, i.e., before the appearance of  $\alpha 6$  subunits, there is a large heterogeneity of the extrasynaptic receptors in cerebellar granule cells (35). A recent study has shown that in adult cerebellar granule cells, the tonic form of inhibition is entirely mediated by BZ insensitive, i.e., receptors most likely formed by the combination of  $\alpha 6\beta 2/3\delta$  subunits (36). This study has shown that mice with genetically ablated  $\alpha 6$  receptors also lack the tonic component of GABA<sub>A</sub> receptor-mediated conductance in cerebellar granule cells. Surprisingly, there is little in the phenotype of the  $\alpha 6$  null mutant to indicate that the loss of this tonic inhibitory activity might have been important for the regulation of granule cell excitability. However, the authors go on to show that the GABA<sub>A</sub> receptor-mediated C1-conductance was fully replaced in

the null mutants by the up regulation of a continuously active K<sup>+</sup> conductance through TASK-1 K-channels, that are not normally expressed in these neurons. This remarkable finding indicates that the tonic conductance generated by extrasynaptic GABA<sub>A</sub> receptors is registered by various feedback mechanisms within the cell, such that in its absence another conductance can take its place to control excitability.

*The  $\delta$  Subunit.* The most compelling evidence to date for the existence of extrasynaptic, subtype specific GABA<sub>A</sub> receptors comes from high-resolution ultrastructural localization studies of  $\delta$  subunits. It has been unequivocally shown that  $\delta$  subunit-containing receptors are not concentrated at GABAergic synapses, but are exclusively present at extrasynaptic somatic and dendritic membranes (37). In a recent high resolution light-microscopy study using a special fixation method, it was possible to show that the diffuse membrane localization of the  $\delta$  subunits did not match the distribution of the synaptic GABA<sub>A</sub> receptor anchoring protein gephyrin (38). There is also a tight association between the  $\alpha 6$  and the  $\delta$  subunits. This has been termed a "receptor partnership", as null-mutant mice for the  $\alpha 6$  subunit are also devoid of  $\delta$  subunits in the cell membrane, although there are normal intracellular levels of  $\delta$  subunit mRNA (22). There are also some specific properties of the  $\delta$  subunit containing receptors that may make it suitable for activation by low concentrations of agonist lingering around in the extracellular space for long periods of time. Receptors containing the  $\delta$  subunit have a 50-fold higher affinity for GABA than other GABA<sub>A</sub> receptors, and they do not desensitize upon the prolonged presence of agonist (39). This would be (40–42) consistent with their continuous activation by low levels of ambient GABA and their participation in tonic inhibition. However, it has to be noted that these functional studies were carried out in recombinant systems expressing the  $\delta$  subunits together with  $\alpha 1$  subunits, which may not be their natural subunit partners in the brain (22,30,43). The  $\delta$  subunit containing receptors are also sensitive to Zn<sup>2+</sup> (39), but insensitive to BZ, as their presence appears to mutually exclude the  $\gamma 2$  subunit that confers high BZ sensitivity (44), and are not modulated by the neurosteroid 3 $\alpha$ ,21-dihydroxy-5 $\alpha$ -pregnan-20-one (THDOC) (45). Surprisingly, in spite of this latter property,  $\delta$  subunit null mutant ( $\delta$ -/-) mice are less sensitive to steroids *in vivo*, and consistent with some compensatory subunit alterations, or a non-exclusive extrasynaptic localization of these receptors in dentate gyrus granule cells (DGGCs), the decay rate of IPSCs in the  $\delta$ -/- mice is faster than in controls (46).

*The  $\alpha 4$  Subunit.* The  $\alpha 4$  subunit containing receptors represent the major diazepam-insensitive GABA<sub>A</sub> receptor class in the forebrain (47). To date there are no anatomical reports identifying the  $\alpha 4$  subunit as being mainly extrasynaptic. Its close association with the  $\gamma 2$  subunit (47) makes it very likely that a considerable fraction of  $\alpha 4$  containing receptors are localized at synapses. However, the  $\alpha 4$  is the subunit most closely related to the  $\alpha 6$  (11–13), and in many brain regions the distribution of its mRNA can be found together with  $\delta$  subunits (13). Therefore, it is very plausible that outside the cerebellum where only negligible amounts of  $\alpha 6$  subunit exist, it is the  $\alpha 4$  subunit that forms the “receptor partnership” with  $\delta$  subunits. Indeed, in DGGCs the levels of  $\alpha 4$  and  $\delta$  subunits were concomitantly elevated following pilocarpine-induced status epilepticus in the rat (48), and co-immunoprecipitation indicated a preferential co-assembly of the two subunits in thalamic neurons (43). A recent study using co-immunoprecipitation with a novel  $\alpha 4$  subunit selective antibody claims that only 7% of the total  $\alpha 4$  subunits were associated with  $\delta$ , and most (33%) were associated with the  $\gamma 2$  subunit (49). According to the same study, approximately one half of the  $\alpha 4$  subunit containing receptors had no association with any  $\gamma$  or  $\delta$  subunits, raising the possibility that  $\alpha 4$  subunits can form GABA<sub>A</sub> receptors solely composed of binary  $\alpha 4$  and  $\beta 1$ – $3$  subunits, or that they may associate with the newly cloned  $\epsilon$ ,  $\pi$ , or  $\theta$  subunits, or with some as of yet unidentified subunits. Regardless of the associations of  $\alpha 4$  subunits, it is clear that this subunit is one of the most plastic GABA<sub>A</sub> receptor constituents in the brain. Its levels increase considerably in various models of epilepsy (48,50,51), following steroid withdrawal (52) without a concomitant increase in the  $\gamma 2$  subunit (53), or after chronic administration of a non-competitive NMDA receptor antagonist (54).

*The  $\alpha 5$  Subunit.* In rodents, this subunit predominates at birth, but declines steadily during the postnatal period (13). Nevertheless, there are some areas in the rodent brain, including the CA1 pyramidal cells of the hippocampus, that contain high levels of this subunit even in adult animals. To date, no ultrastructural studies have been carried out on the synaptic or extrasynaptic localization of the  $\alpha 5$  subunits. However, a high-resolution light microscopy method was able to distinguish between synaptic receptors as punctae and possible extrasynaptic receptors as being homogeneously distributed over the cells’ surface (38), and has identified the  $\alpha 5$  subunits as non-aggregating, and thus unlikely to be highly concentrated at synapses (55).

Biophysical studies of the  $\alpha 5\beta 3\gamma 2L$  type receptors expressed in L929 fibroblasts have shown long closed states characteristic of desensitization, and a marked voltage-dependency for the deactivation of the receptors (56). Interestingly, these receptors show some remarkable plasticity following status epilepticus induced by pilocarpine (57).

The presence of significant concentrations of GABA in the extracellular space necessary and sufficient for the activation of extrasynaptic GABA<sub>A</sub> receptors may alter the state of synaptic receptor channels by driving them into an absorbing desensitized state thus changing the efficacy of synaptic transmission (58). However, it is not necessary to postulate that GABA levels in the extracellular space need to be quite high. In addition to the activation of extrasynaptic GABA<sub>A</sub> receptors by ambient GABA levels or by GABA “overspilled” from neighboring synapses, spontaneous openings of GABA<sub>A</sub> receptor channels in the absence of any agonist have been reported to occur in CA1 pyramidal cells (59). Nevertheless, this peculiar mode of activation of tonic GABA<sub>A</sub> receptor openings may be altered by intra- or extracellular modulators of the receptors.

*Future Directions for Separating between the Phasic and Tonic Modes of GABA<sub>A</sub> Receptor Activation.* According to the few physiological and morphological studies, receptors generating tonic or phasic inhibition seem to have different molecular structures, subcellular distributions, kinetics and pharmacological properties. The ability to selectively modify the receptors responsible for either of these inhibitions holds tremendous potential for altering different properties of neural networks including those underlying higher cognitive functions. The authors of a recently published study report that the two competitive GABA<sub>A</sub> receptor antagonists bicuculline and gabazine (SR-95531) may distinguish between phasic and tonic GABA<sub>A</sub> conductances. According to this study, gabazine blocks IPSCs only (phasic conductance), while bicuculline reduces both phasic and tonic currents (60). Most experiments of this study were carried out in primary cell cultures, and since gabazine was shown in a different study using slices to be effective against the tonic current in cerebellar granule cells (36), the general validity of the distinct effects of the two competitive antagonists remains to be further determined. Nevertheless, the question about specific agonists and antagonists of the two types of conductance will have to be addressed systematically by probing the distinct pharmacological properties of synaptic and extrasynaptic receptors, and by identifying compounds that



preferentially alter one but not the other type of inhibition (Table I). A large variety of drugs exert their influence through GABA<sub>A</sub> receptors. Some of them are extensively used in the clinic to induce anesthesia, to treat depression, anxiety or to stop seizures (11–13). The effects of some of these drugs, such as the anxiolytic, anticonvulsant, sedative-hypnotic BZ or some general anesthetic steroids, are known to depend on the subunit composition of GABA<sub>A</sub> receptors. For example, only  $\gamma 2$  subunit-containing GABA<sub>A</sub> receptors have high affinity for BZ, as opposed to those containing the  $\gamma 1$ ,  $\gamma 3$  or  $\delta$  subunits (12,13). Furthermore, even within the  $\gamma 2$  subunit-containing receptors, the identity of the  $\alpha$  subunit also plays an important role in determining the sensitivity for certain BZ agonists. For example,  $K_i$  values of zolpidem for displacing Ro-15-1788 from GABA<sub>A</sub> receptors with  $\alpha 1\beta 3\gamma 2$ ,  $\alpha 2\beta 3\gamma 2$ ,  $\alpha 3\beta 3\gamma 2$  and  $\alpha 5\beta 3\gamma 2$  subunit composition are approximately 20, 450, 400 and 15000 nM, respectively (61). As cerebellar extrasynaptic receptors may also contain the  $\alpha 6$  subunit, associated with the  $\delta$  subunit, it might be possible to inhibit such receptors specifically with furosemide, a known antagonist of  $\alpha 6$  subunit containing receptors (33). There are several other subtype-selective modulators of GABA<sub>A</sub> receptor function, but the effects of such highly specific GABA<sub>A</sub> receptor modulators have not yet been studied on the control of tonic vs phasic inhibition in cortical networks. Nor has it been investigated how such drugs would influence network oscillations thought to underlie higher cognitive functions. One of the fundamental goals of future research should be to identify specific pharmacological agents acting exclusively on the two types of inhibition. Once identified, these drugs might be used for studying the roles of tonic and

phasic inhibitions in controlling the excitability of cortical networks including physiological oscillations and the pathological propagation of epileptiform activity.

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**Table I.** Pharmacological Specificity of GABA<sub>A</sub> Receptor Subunits Responsible for the Tonic Conductance and Most Likely Found Extrasynaptically

Subunit	Specific Pharmacology
$\alpha 4/\alpha 6$	BZ insensitive Inhibited by furosemide Inhibited by $La^{3+}$
$\delta$	High $Zn^{2+}$ sensitivity High affinity for GABA Little or no desensitization
$\alpha 5$	Low sensitivity to THDOC (steroid) Low sensitivity to zolpidem & alpidem High sensitivity to L655,708 High sensitivity to Ro 15-4513 & B-CCM

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