Activation of GABA$_A$ Receptors: Views from Outside the Synaptic Cleft

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Some GABA$_A$ receptors (GABA$_A$Rs) are activated by low transmitter levels present in the extracellular space and generate an uninterrupted conductance referred to as "tonic." This tonic conductance is highly sensitive to all factors regulating the amount of GABA surrounding the neurons. Only a few GABA$_A$Rs with particular subunit combinations are well suited to mediate the tonic conductance. These same receptors constitute important and specific targets for various endogenous and exogenous neuroactive compounds and possible therapeutic targets.

Introduction

Chemical communication within the body occurs at three different temporal and spatial domains: (1) the endocrine system relies on the bloodstream to carry messengers relatively slowly but in a spatially unrestricted manner, (2) volume transmission through the extracellular space is much faster but can only reach neighboring cells by diffusion of transmitter over hundreds of microns, and (3) synaptic transmission, which is the fastest, but requires specialized structures (synapses) between two communicating cell partners separated only by 20 nm. In the mammalian CNS, GABA synapses were long known to generate fast and precisely timed inhibitory activity in the form of inhibitory postsynaptic currents (IPSCs or phasic inhibition) (Mody et al., 1994; Farrant and Nusser, 2005). But over the last decade, diffusional inhibitory transmission mediated by GABA$_A$Rs located outside the synapses and activated by the GABA levels present in the extracellular space has triggered a great deal of interest (Mody, 2001; Semyanov et al., 2004; Farrant and Nusser, 2005; Cavelier et al., 2005; Semyanov, 2005; Vizi and Mike, 2006; Orser, 2006). This form of inhibition is generally referred to as tonic inhibition, while the conductance generated by the GABA$_A$Rs is known as tonic conductance. Such conductance has been found in a large variety of principal neurons and interneurons, including those found in the cerebellum, cortex, hippocampus, thalamus, and spinal cord. This review focuses on some new developments and technical issues related to the tonic inhibition of adult neurons and attempts to call out for a standardized approach for its recording and measurement.

Which GABA$_A$ Receptors Mediate the Tonic Conductance?

The GABA$_A$Rs responsible for mediating a current that is "always on" should fulfill certain criteria. First and foremost, the receptor should have a sufficiently high GABA affinity to be activated by the near micromolar GABA concentrations present in the extracellular space (Nyitrai et al., 2006). This is in sharp contrast to the GABA$_A$Rs situated at synapses that need not have a high GABA affinity to react rapidly to fast rises in cleft GABA concentrations to 1.5–3 mM that decay within a few hundred microseconds (Mozrzymas et al., 2003). Establishing the precise affinity for GABA of the native GABA$_A$Rs, whether synaptic or not, is not an easy task. Specific interactions with other neuronal proteins and cell-specific posttranslational modifications can make receptors found on the surface of neurons to function unlike those studied in heterologous expression systems, where GABA affinity can be easily determined. The contributions to the phasic and tonic currents of a multitude of GABA$_A$Rs with different GABA affinities may make it even more difficult to determine the role of a receptor with a specific subunit composition. To make things more complicated, some GABA$_A$Rs can be tonically active in the absence of any ligand (McCartney et al., 2006), and thus their contribution to the tonic current may artificially increase the apparent GABA affinity of the combined pool of receptors generating the tonic conductance. A second important factor to consider in the tonic activation of GABA$_A$Rs is desensitization. This is a common property of ligand-gated ion channels characterized by long periods of closed (nonconducting) states while the agonist is still bound to the receptor. Considering the single-channel conductance to be the same, a fewer number of nondesensitizing receptors would be needed to generate a tonic conductance of a given size, but the simultaneous openings of a much larger number of desensitizing receptors could also sum to produce a tonic current of similar magnitude. But clearly, receptors with high GABA affinity and little desensitization would be better suited to mediate a tonic conductance. Thus far, only four types of heteropentameric GABA$_A$R assemblies containing either the δ, α5, or ε subunits or receptors containing only αβ subunits

have been shown to mediate individually, or in combination, the tonic conductance of a variety of central neurons. Considering the various α, β, and γ subunits that can assemble with these specific subunits, probably no more than a dozen GABA$_A$R subunit combinations mediate the tonic conductance in the brain.

The GABA$_A$Rs containing δ subunits (GABA$_A$Rδ) in combination with either α4 or α6, and β2 or β3 subunits satisfy both the high affinity and limited desensitization criteria. Their half-maximal activation by GABA (EC$_{50}$) is in the tens of nanomolar range, well within the range of GABA found in the extracellular space (Saxena and Macdonald, 1994; Wallner et al., 2003). The GABA$_A$Rδs also have a low degree of desensitization in the continuous presence of agonist (Haas and Macdonald, 1999; Wohlfarth et al., 2002; Bianchi and Macdonald, 2003). In addition, these subunits have two other interesting properties that aid their function as one of the prime mediators of tonic inhibition throughout the brain. The first is their extra- and perisynaptic localization. The GABA$_A$Rδs are scattered over the surface of cerebellar granule cells (Nusser et al., 1998) at locations far from the synapses (extrasynaptically). In the granule cells of the dentate gyrus, another area of the brain with high levels of δ subunits, the same receptors are localized somewhat closer to the outside edges of synapses (perisynaptically); this is an ideal location to sense GABA spilled over following vesicular release from nearby boutons or to be activated by the ambient levels of GABA present in the extracellular space (Wei et al., 2003). Their second property is the inefficiency of coupling GABA binding to channel gating, i.e., GABA is a low-efficacy agonist at δ subunit-containing GABA$_A$Rs. It is not intuitively obvious why GABA should be a low-efficacy agonist at δ subunit-containing GABA$_A$Rs while their affinity for GABA is very high. But this interesting property means that the predominant mechanism for enhancing the function of these receptors may be through increasing the efficacy of GABA as an agonist instead of increasing their already exceptionally high affinity for GABA. This property may be critical in mediating the actions of potent endogenous modulators of GABA$_A$R function, i.e., 3α-hydroxy ring A-reduced pregnane steroids (neurosteroids), the brain-derived metabolites of ovarian and corticosteroids (Majewska et al., 1986; Belelli and Lambert, 2005). Neurosteroids enhance the efficacy of GABA at GABA$_A$Rδ (Wohlfarth et al., 2002; Bianchi and Macdonald, 2003). The low efficacy of GABA at these receptors also means that there might be other compounds that are more efficacious than GABA itself. The GABA agonist gaboxadol (4,5,6,7-tetrahydroisoxazolo-[5,4-c]pyridine-3-ol, or THIP) is such a compound (Brown et al., 2002). The overall properties of GABA$_A$Rδs make them ideal for mediating a tonic current activated by GABA circulating in the extracellular space. Indeed, physiological/pharmacological approaches and the use of null mutants have unequivocally shown that in several cell types of the mammalian CNS, including the cerebellar granule cells (Stell et al., 2003), dentate gyrus granule cells (Stell et al.,

2003), thalamic neurons (Cope et al., 2005; Bright et al., 2007), layer 2/3 pyramidal cells (Drasbek and Jensen, 2006), and interneurons of the dentate molecular layer (Glykys et al., 2007), these receptors predominate in generating a tonic conductance.

In addition to the GABA$_A$Rδs, GABA$_A$Rα5 has also been shown to be critically involved in mediating tonic currents in CA1 and CA3 pyramidal cells (Caraiscos et al., 2004; Glykys and Mody, 2006, 2007; Cheng et al., 2006; Prenosil et al., 2006) and in cortical layer 5 pyramidal cells (Yamada et al., 2006). The GABA$_A$Rα5s also have a high GABA affinity and relatively low desensitization (Burgard et al., 1996; Caraiscos et al., 2004). Receptors devoid of a third type of subunit, i.e., only containing α and β subunits that are highly sensitive to Zn^{2+}, have been shown to contribute to the tonic current recorded in hippocampal neurons (Mortensen and Smart, 2006). Moreover, the ε subunit-containing GABA$_A$Rs found on CA3 pyramidal cells may not even require GABA for ligand-independent openings that could underlie a tonic current (McCartney et al., 2006).

Many different GABA$_A$R types capable of generating a tonic conductance may be simultaneously expressed on the surface of a neuron. However, only some types may be active during a given condition. As conditions change around the neurons, for example through alterations of GABA levels (Scimemi et al., 2005), the presence of modulators, localization of the GABA source, developmental alterations, or other factors, different fractions of tonically active GABA$_A$Rs may be contributing to the total measured tonic current. Figure 1 illustrates how the fractional contribution of various GABA$_A$R assemblies to the total tonic conductance may obscure some, or nearly all, of the effects of a δ subunit-specific modulator (ethanol) on the total tonic conductance recorded in three types of hippocampal neuron. This situation presents interesting challenges for developing pharmacological approaches to a cell- or brain-region-specific modulation of tonic inhibition.

Technical Issues Related to the Recording and Measurement of Tonic Inhibition

Under the right conditions, a tonic inhibitory conductance mediated by GABA$_A$Rs is easy to record in a variety of preparations. Just what exactly are the "right" conditions? Most preparations amenable for electrophysiological recordings use nerve cells removed from their natural environment—the brain. The existence of a tonic conductance in cerebellar granule cells recorded in vivo (Chadderton et al., 2004) proves that the tonically active GABA$_A$R-mediated conductance is not an artifact of in vitro preparations. Nevertheless, the conditions used to record tonic conductances mediated by GABA$_A$Rs in vitro are still as diverse as the investigators performing the recordings.

The amount of GABA present in the extracellular space of an in vitro preparation mostly depends on the volume in which GABA is dissolved (the extent of the extracellular space). This space varies depending on the region of the brain, the age of the animal from which the tissue was obtained, and the manner in which the slices are kept for
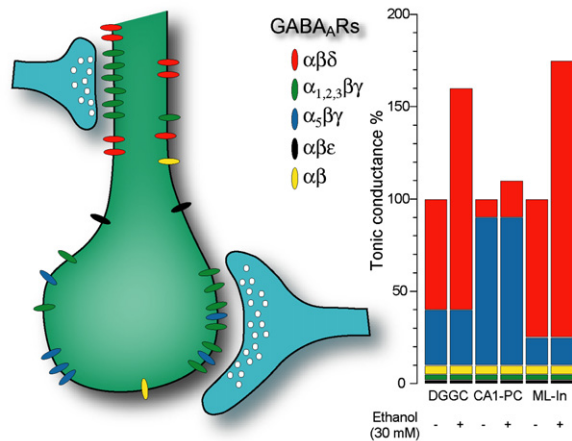
Figure 1. The Cartoon Illustrates the Various Types of GABA$_A$ Receptor Assemblies Known to Be Involved in Generating Tonic Conductances

The different subunit-containing receptors are denoted by different colors. According to the relative distribution of these receptors on the cell surface and the specific conditions leading to their activation, in any given cell a mixture of receptors may be responsible for generating a compound tonic conductance. The right panel illustrates this as a hypothetical graph in three different types of hippocampal neurons: a dentate gyrus granule cell, a CA1 pyramidal cell, and a dentate gyrus molecular layer interneuron. Depending on the ratios of specific receptors contributing to the tonic current, a modulator that is specific only to certain subunits (e.g., ethanol for the δ subunit-containing receptors) (Mody et al., 2007), may have its effects obscured by the contribution to the tonic current of receptors insensitive to the compound.

recording (interface or submerged). It also depends on the level of tissue oxygenation. Hypoxia shrinks the extracellular space (Nicholson and Sykova, 1998), which may lead to an increased GABA concentration in this compartment. In submerged slices (used for visualized patch-clamp recordings), tissue O$_2$ tension needs to be increased by enhancing the flow rate of the oxygenated solution to produce oscillations (Hajos et al., 2004) readily observed in the brain or in slices maintained in an interface-type chamber. Such increased perfusion rate may wash away the ambient transmitter, leading to a reduction or even elimination of the tonic current, particularly in cells on the surface of the slices.

Other problems are posed by the temperature at which the recordings are done, by the age of the animals from which slices are prepared, and the general health of the tissue. At room temperature, the efficiency of amino acid transporters is decreased (Asztely et al., 1997; Mitchell and Silver, 2000). As GABA transporters are critical for controlling GABA levels in the extracellular space (see below), a diminished GABA uptake at room temperature may be sufficient to activate the tonic conductance.

Slices used for recordings are mostly prepared from young or "juvenile" mice or rats (<21 days of age). At this age, many of the neurotransmitter systems, their receptors, and second messengers have not yet matured, thus making difficult a fair comparison with studies carried out in fully mature (>60 days old) animals. A high frequency

of spontaneous firing of GABAergic interneurons or a large number of damaged glial cells or neurons could also contribute to raising ambient GABA levels. The amount of GABA present in the extracellular space may be standardized by blocking the GABA-degrading enzyme GABA-transaminase with vigabatrin, by adding GABA uptake blockers, or GABA itself (see below) to the extracellular solution. Unfortunately, none of the approaches used in vitro will fully mimic the conditions found in the intact brain.

Compared to the conditions of recording, there is considerably more agreement in the literature on the method of measurement of the tonic GABA-generated conductance. One usually measures the difference current in the absence and the presence of a GABA$_A$R blocker. In practice, the mean holding current during a given segment of arbitrary length is measured in the presence of the antagonist and is compared to the mean current recorded during several segments of equal length recorded prior to the antagonist administration (Nusser and Mody, 2002). We have recently refined this method to separately measure tonic and phasic inhibitions during consecutive arbitrary epochs (Glykys and Mody, 2007). This method allows the continuous monitoring of tonic and phasic conductances and any potential correlation between the two. When comparing tonic conductances between different cell types, it is a good practice to express it as a normalized value (in pS/pF) that accounts for the surface of the cell that is electrically controlled.

Very low amplitude tonic currents (a few pA) make it impractical to measure absolute changes. In such instances it may appear useful to measure the root-mean-squared (RMS) value of the current or the change in the RMS noise (which is equivalent to measuring a change in the variance) of the current.

The RMS for a number of n successive digitized data points x_t is obtained as

$$RMS = \sqrt{\left(\frac{1}{n}\sum_{n=1}^{i} x_i^2\right)}$$

Then, the relationship between the RMS and the mean current

$$I_m = \frac{1}{n}\sum_{n=1}^{i} x_i$$

and its population variance

$$\sigma^2 = \frac{1}{n}\sum_{n=1}^{i}(x_i - I_m)^2$$

(also referred to as "RMS noise") becomes

$$RMS^2 = I_m^2 + \sigma^2$$

Because the openings of the active channels should sum to generate the current, a larger current should be characterized by a larger variance. Yet, specific alterations in channel properties (see Figure S1 available online),

including their conductance, could lead to a change in I_m (and thus RMS), but not in σ^2. Caution should be exercised when judging changes in baseline currents simply based on the change in RMS noise alone.

The type of GABA$_A$R blocker and its concentration used to reveal the tonic conductance should also be carefully chosen. As GABA$_A$Rs mediating tonic and phasic conductances most likely have different GABA affinities (Stell and Mody, 2002), care must be taken to use antagonists at sufficiently large concentrations to block both types of GABA$_A$R. Moreover, for the channels that are tonically active in the absence of GABA (e.g., the GABA$_A$Rϵ), bicuculline may be the only drug of choice, as gabazine does not block these receptors (McCartney et al., 2006). Bicuculline, however, by blocking SK-type K$^+$ channels (Khawaled et al., 1999), could confound the measurement of tonic inhibition. Picrotoxin may affect GABA$_A$Rs specific only to certain cell types (Semyanov et al., 2003), as well as Cl$^-$ channels not operated by GABA$_A$Rs.

The Tonic Conductance and the Composition of the Artificial Cerebrospinal Fluid

The CSF and the extracellular fluid of the brain are not just simple mixtures of salts in solution that are commonly used to prepare the artificial CSF (aCSF) for in vitro recordings. Additions of ascorbic acid or Na-pyruvate to reduce oxidative stress and to enhance slice viability are common, but real CSF ingredients such as amino acids and other neuroactive compounds are invariably left out. In order for "tonic" inhibition to be always on, the continuous presence of the agonist (in this case GABA) is required around the cells. Neurons in brain slices do release GABA, as it is clear from the frequent GABAergic inhibitory postsynaptic events that can be recorded without any stimulation. But these events are generated by vesicular GABA release into the synaptic cleft, where on the postsynaptic side there are dozens of receptors eager to bind the excess transmitter. In contrast, the receptors responsible for the tonic inhibition are mostly found just outside (perisynaptically) or far away from synapses (extrasynaptically). The open environment of a brain slice perfused with aCSF at rates of 1–10 ml/min may not withhold sufficient levels of transmitter to activate receptors at a considerable distance from the release sites. In some cases, not even this "harsh" washing can prevent GABA from reaching extrasynaptic sites. This happens at specialized synapses where glial ensheathing or glomerular structure might prevent rapid diffusion, as in cerebellar granule cells and dLGN thalamic relay neurons where tonic GABAergic inhibition can be recorded without the need of adding GABA to the aCSF. In other parts of the brain, tonic currents can be observed under some conditions without supplementing the aCSF with GABA. Tonic GABA$_A$ conductance recorded under such conditions could be considered "physiological," but it is debatable whether the sources of GABA are "physiological" in such slice preparations. It is equally disputable whether it is "unphysiological" to provide isolated and perfused brain tissue with

natural ingredients of the brain's extracellular space by including them in the aCSF. Today, nobody in their right mind would consider Ca to be an "artificial" additive of the aCSF (or Ringer's solution). Yet, Sydney Ringer himself didn't think of including Ca in his famous solution until the day a solution prepared not from distilled water, but from water supplied by the New River Water Co., containing 38.3 ppm Ca (almost 1 mM), sustained the beating of a frog's heart maintained in vitro (Miller, 2004).

Supplementing the aCSF with amino acids and other neurotransmitters is complicated by the discrepancies in the measurements of amino acid concentrations present in the CSF and extracellular space (Nyitrai et al., 2006). Furthermore, different brain areas have different extracellular levels of amino acids, and local activity-dependent changes can also offset the measurements. A meeting of microdialysis experts and slice physiologists may be a good way to start developing a consensus on this topic.

The Role of GABA Uptake and the Source of GABA

The concentration of a diffusing substance released from a source into the extracellular space will depend on the extracellular space volume fraction, tortuosity, and the presence of buffering systems (Nicholson and Sykova, 1998). The tortuosity has both a geometric and a viscous component (Rusakov and Kullmann, 1998) that will slow the diffusion of neurotransmitter, in this case GABA in the extracellular space. The buffering systems consist of transporters, receptors, and other GABA binding sites. The most effective component of these buffering systems is the GABA transporter that not only passively binds GABA but also actively removes it from the extracellular space. The GABA transporters are high-affinity Na$^+$/Cl$^-$-dependent membrane translocators of GABA (Chen et al., 2004). There are three transporters specialized in GABA transport (SLC6A1 [GAT-1], SLC6A13 [GAT-2], and SLC6A11 [GAT-3, homologous to mouse mGAT-4]) with specific regional and cellular distribution in the brain (Conti et al., 2004). GAT-1, the most prevalent GABA transporter, has a relatively high density on the surface of the neurons. There are around 1000 mGAT-1 molecules/μm^2 in the neighborhood of GABA synapses (Chiu et al., 2002). Considering the few thousand GABA molecules in the synaptic cleft corresponding to a GABA concentration of 1.5–3 mM (Mozrzymas et al., 2003) and a maximum of hundreds of GABA$_A$Rs, the uptake molecules should bind the largest fraction of the released GABA. The binding and subsequent removal of GABA makes the uptake system the most effective regulator of GABA concentrations in the extracellular space and thus it should be considered one of the most important regulators of the tonic conductance.

Indeed, tonic inhibition is exquisitely sensitive to the amount of GABA uptake (Nusser and Mody, 2002; Semyanov et al., 2003). Accordingly, GAT-1-deficient mice have much higher levels of tonic conductance (Jensen et al., 2003), which may be the cause of their phenotype of tremor, ataxia, and nervousness (Chiu et al., 2005). In addition to GAT-1, GAT2/3 may be involved in soaking up

GABA in the neocortex to limit the activation of extrasynaptic receptors (Keros and Hablitz, 2005). As under certain conditions the GABA uptake system may also function in reverse, it can become a source of GABA rather than a sink (Richerson and Wu, 2003). It has been suggested that the very source of GABA for the large tonic current seen in some recorded neurons is the release through the reversed transport from the recorded cells themselves (Richerson and Wu, 2003). In contrast, the several-fold increases in tonic currents recorded in GAT-1-deficient mice (Jensen et al., 2003; Chiu et al., 2005) tend to argue against the role of a reversed GABA transport through GAT-1 as the source for GABA. Nevertheless, GABA release through reversal of uptake may constitute an important source of extracellular GABA, particularly under specific experimental conditions when cells are overly depolarized or when the Na^+ concentration inside the cells is altered. The uptake of amino acids in the brain is highly temperature sensitive (Asztely et al., 1997; Mitchell and Silver, 2000), with a Q_{10} for the mGAT-4 as high as 4.3 (Karakossian et al., 2005), and many other additional factors, such as posttranslational modifications, regional differences, tissue oxygenation, and developmental stage, can also influence GABA transporter activity. Therefore, the contribution of the GABA transporter to the tonic conductance should be determined for each preparation to clarify possible discrepancies between data obtained in different labs. Since uptake systems active in a brain slice can reduce GABA levels even when GABA is included in the aCSF (Glykys and Mody, 2006) and because GABA uptake has the lion's share of control over the tonic conductance, the modulation of the uptake should be addressed before suspecting the modulation of the receptors responsible for generating the tonic current (Mody et al., 2007). Eventually it will be necessary to determine the free GABA concentrations present in slices of various brain regions, with and without added GABA, at various depths under the slice surface as well as under high or low levels of neuronal activity. In the absence of fast and reliable GABA-sensing devices, the ingenious method using outside-out patches (Isaacson et al., 1993) containing $GABA_A$Rs may be a way to systematically measure slice GABA levels that could originate from diverse sources. Astrocytic release (Kozlov et al., 2006), reversal of GABA transporter (Richerson and Wu, 2003), and nonvesicular release, as well as action potential-mediated release (Attwell et al., 1993; Brickley et al., 1996; Rossi et al., 2003; Bright et al., 2007; Glykys and Mody, 2007) have all been proposed to contribute to extracellular GABA in slices. Early on in development in cerebellar granule cells the tonic current depends on the firing of action potentials (Kaneda et al., 1995; Brickley et al., 1996), but in adult granule cells this source of GABA is replaced by action potential-independent mechanisms (Wall and Usowicz, 1997; Rossi et al., 2003). But, since the inhibitory inputs onto cerebellar granule cells form a unique synaptic structure, the glial ensheathed glomerulus, transmitter release, diffusion, and overspill may be unique to this highly specialized synapse.

The rest of the brain has less specialized GABA synapses than those on cerebellar granule cells. Generally, there seems to be a correlation between the magnitudes of tonic and phasic inhibition, indicating that there might be a common source of GABA for the activation of the two types of conductances. In mouse spinal cord dorsal horn lamina II neurons, the phasic and tonic currents are correlated (Ataka and Gu, 2006), but this is not the case in the magnocellular vasopressin and oxytocin secretory neurons, where the local glial GABA transporter GAT-3 controls the level of tonic conductance (Park et al., 2006). At the synapses of dLGN thalamic relay neurons, tonic inhibition depends on the global level of inhibitory activity and vesicular release (Bright et al., 2007). We recently established high temporal correlations between the two types of inhibitory activity under conditions of both increases and decreases of vesicular GABA release (Glykys and Mody, 2007). If vesicular GABA release is proven to be the source of GABA responsible for both types of inhibition, the analogy between tonic inhibition and the sound of a distant orchestra playing at the synapses (Soltesz and Nusser, 2001) will prove to be correct. If, however, most of the GABA in the extracellular space originates from sources other than synaptic vesicles, then tonic inhibition might be playing a different tune than the synaptic symphony. In the intact brain, it is very likely that various physiological and pathological conditions can shift the balance between the many possible sources and sinks of GABA controlling tonic inhibition.

Functional and Therapeutic Perspectives on Tonic $GABA_A$ Inhibition

During ontogeny and in the absence of synaptic contacts, diffusional neurotransmission is the norm, and much has been written about the role of GABA in the development and maturation of neurons born both in the developing and the adult CNS (Owens and Kriegstein, 2002; Ge et al., 2007). As the topic of this review is the tonic $GABA_A$R-mediated conductance found in fully developed neurons, the role of the tonic conductance during development and maturation will not be discussed here. It is not difficult to see how a steady conductance of a considerable magnitude can affect neuronal excitability. When compared to the charge carried by the phasically active (synaptic) channels, the tonically active receptors invariably come out on top by a margin of 3:1 to 5:1 (Mody and Pearce, 2004; Cavelier et al., 2005). This is not to say that the phasic conductance does not have a role in controlling excitability, but its actions have to be considered in a highly timed fashion depending on the input received by the interneurons as well as by their targets. In contrast, the presence of an uninterrupted GABA conductance will control the overall gain of the neuronal input-output (Mitchell and Silver, 2003; Chadderton et al., 2004; Semyanov et al., 2004; Cavelier et al., 2005).

Uninterrupted as the tonic conductance may be, this property should not be taken to mean that the conductance remains constant in its magnitude over time.

Fluctuations in its levels secondary either to changes in the concentrations of GABA surrounding the cells or in the number or properties of the GABA$_A$Rs responsible for the conductance can have profound effects of neuronal excitability. We are just beginning to explore the functional consequences of the changes in δ subunit-containing GABA$_A$Rs during the ovarian cycle (Maguire et al., 2005; Lovick, 2006), their altered expression in certain models of epilepsies (Peng et al., 2004; Zhang et al., 2007), or of their variants as the genetic basis for certain human epilepsies (Mulley et al., 2005). The high sensitivity of the tonically active GABA$_A$Rs to stress-related neurosteroids (Stell et al., 2003) and to sobriety-impairing concentrations of ethanol (Mody et al., 2007) will open new opportunities for understanding the effects of stress and ethanol on the brain. There might be potential clinical use of gaboxadol, a compound with high specificity and efficacy for GABA$_A$Rδ, as a novel hypnotic that enhances slow wave sleep (Wafford and Ebert, 2006). Gaboxadol has also been proposed for the treatment of premenstrual dysphoric disorder (Maguire et al., 2005), and these may be but the first steps in uncovering the clinical usefulness of a new class of compounds enhancing the function of tonically active GABA$_A$Rs.

The critical role of the tonically active α5 subunit-containing GABA$_A$Rs in learning and memory and cognition has been highlighted by numerous pharmacological and mouse knockout studies (Caraiscos et al., 2004; Dawson et al., 2006). L655,708 is an imidazo[1,5-a]benzodiazepine that is a selective and high-affinity (K$_d$ $\sim$2.5 nM) ligand for GABA$_A$Rα5 (Quirk et al., 1996). Together with other related α5 subunit-selective inverse agonists such as RY80 (Liu et al., 1996) or α5IA [i.e., 3-(5-methylisoxazol-3-yl)-6-[(1-methyl-1,2,3-triazol-4-yl)methyloxy]-1,2,4-triazolo[3,4-a]phthalazine], these compounds have been suggested for use against cognitive dysfunction based on their specific actions of enhancing the excitability of CA1/3 hippocampal neurons by presumably reducing the tonic inhibition mediated by GABA$_A$Rα5 (Atack et al., 2006; Dawson et al., 2006). In addition, the gene encoding the α5 subunit (GABRA5) figures prominently among the several candidate genes for schizophrenia, and more recently among those linked to bipolar disorder and depression (Kato, 2007). The disproportionately large charge carried by tonically active GABA$_A$Rs compared to that mediated by the phasic conductance makes tonic inhibition the preferred site of action of several sedative-hypnotic drugs (Orser, 2006). Interestingly, the amnestic effects of etomidate, but not its sedative-hypnotic actions, can be attributed to the enhancement of the tonic conductance mediated by GABA$_A$Rα5 of hippocampal pyramidal cells (Cheng et al., 2006).

Another potential intervention for controlling the amount of the tonic GABA$_A$R-mediated conductance is regulating the levels of extracellular GABA through altering the function of GABA transporters. Reducing the function of GAT-1 by tiagabine is already an effective therapy for epilepsy (Gether et al., 2006) and may prove to be a promising target for anxiety disorders (Schwartz and Nihalani, 2006). It is important to note, however, that increasing extracellular GABA levels either by inhibiting GABA uptake (e.g., by tiagabine) or by reducing the degradation of GABA by inhibiting GABA-transaminase (e.g., by vigabatrin) will have a combined effect consisting of an increased tonic conductance mediated by ionotropic GABA$_A$Rs and an enhanced activation of the metabotropic GABA$_B$Rs. Indeed, the potential effectiveness of tiagabine in treating cocaine addiction is most likely due to an effect of GABA on GABA$_B$Rs (Sofuoglu and Kosten, 2005). The most serious side-effect of vigabatrin therapy is visual dysfunction including retinal atrophy, but the underlying mechanism is not well understood, and no link to any specific GABA system has been established (Wheless et al., 2007). In contrast to the beneficial effect of tiagabine in epilepsy, some antiepileptic drugs, such as valproate, may actually decrease the function of GABA transporters (Whitlow et al., 2003). Interestingly, GAT-1 knockout mice exhibit a constant and significant increase in the tonic GABA$_A$ conductance, but no sign of an elevated GABA$_B$R activation (Jensen et al., 2003). These mice have motor disorders, including gait abnormality, constant 25–32 Hz tremor, reduced rotarod performance, and reduced locomotor activity in their home cage (Chiu et al., 2005).

In spite of the recent surge of interest in the identity and function of tonically active GABA$_A$Rs, there are quite a number of important unresolved issues surrounding the tonic GABA$_A$ conductance that have yet to be addressed experimentally. For example, we do not know how most receptors responsible for this conductance are kept away from synapses, ensuring their confinement to extrasynaptic or perisynaptic sites. There are no reports on the effects of posttranslational modifications of the tonically active receptors. We know little about their turnover rates or about the regulation of their expression on the cell surface by endo- or exocytosis. Not much is known about functional changes in these receptors during development or aging. Furthermore, although the experimental techniques are in place, we are still in the dark about the precise GABA affinity and GABA efficacy of these receptors when found in their natural habitat, i.e., on neurons of the CNS. Getting the answers to some of these questions and a continued high level of interest in GABA$_A$R-mediated tonic conductances will lead us onto an exciting scientific journey to unexplored territories where we can begin elucidating how critical neuroactive compounds affect the brain, thus gaining insights into the mechanisms of several debilitating neurological and psychiatric disorders.

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