A new meaning for "Gin & Tonic": tonic inhibition as the target for ethanol action in the brain

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Abstract

Gamma-aminobutyric acid (GABA) is the main chemical inhibitory neurotransmitter in the brain. In the central nervous system, it acts on two distinct types of receptor: an ion channel, that is, an "ionotropic" receptor permeable to Cl^- and HCO_3^- (GABA$_A$ receptors [GABA$_A$Rs]) and a G-protein coupled "metabotropic" receptor that is linked to various effector mechanisms (GABA$_B$ receptors). This review will summarize novel developments in the physiology and pharmacology of GABA$_A$Rs, specifically those found outside synapses. The focus will be on a particular combination of GABA$_A$R subunits responsible for mediating tonic inhibition and sensitive to concentrations of ethanol legally considered to be sobriety impairing. Since the same receptors are also a preferred target for the metabolites of steroid hormones synthesized in the brain (neurosteroids), the ethanol-sensitive tonic inhibition may be a common pathway for interactions between the effects of alcohol and those of ovarian and stress-related neurosteroids. © 2007 Elsevier Inc. All rights reserved.

Keywords: GABA$_A$Rs; Ethanol; Inhibition; Tonic inhibition; Neurosteroids

Introduction

Cell-to-cell chemical communication in the body can take three forms, each having various temporal and spatial limitations: (1) the relatively slow but spatially unrestricted neuroendocrine secretion, (2) the much faster volume transmission that reaches neighboring cells by diffusion of transmitter over hundreds of μm in the extracellular space, and (3) the ultra-fast synaptic transmission that requires specialized structures (synapses) between two communicating cell partners separated roughly by 20 nm. For the brain's principal chemical inhibitory transmitter gamma-aminobutyric acid (GABA), fast synaptic transmission has been long thought to be the sole mechanism for communication between cells. More recently, the nonsynaptic localization of the metabotropic GABA$_B$ receptors and of a certain type of the ionotropic GABA$_A$ receptors (GABA$_A$Rs) has triggered a great interest in the diffusional inhibitory transmission (Cavelier et al., 2005; Farrant and Nusser, 2005; Kullmann et al., 2005; Semyanov et al., 2004; Vizi and

Mike, 2006). To distinguish between the activation of GABA$_A$Rs at synapses and of those on the outside or on the periphery of synapses one refers to phasic and tonic inhibitions, respectively. The fast and local and slower but distant modes of GABAergic signaling is one of the principal reasons for the diversity of GABAergic action in the brain (Mody, 2001; Mody and Pearce, 2004). Many excellent reviews have been written on tonic inhibition and its role in the control of neuronal excitability (Cavelier et al., 2005; Farrant and Nusser, 2005; Kullmann et al., 2005; Semyanov et al., 2004; Vizi and Mike, 2006), therefore, our review will focus only on some methodological aspects related to its measurement. The main topic of the present review is the modulation of tonic inhibition by ethanol and by various endogenous steroid-hormone derived substances that are highly relevant to everyday life.

The δ subunit-containing GABA$_A$Rs

GABA$_A$Rs are members of the superfamily of Cys-loop ligand gated ion channels in which five protein subunits (usually different proteins, and thus the name heteropentameric receptors) co-assemble to form a central aqueous pore through the lipid bilayer of the cell membrane (Sine and Engel, 2006). Ligand binding produces

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a conformational change in the receptor, and the central ion pore opens to allow the flow of ions. The channels open and close extremely fast until the ligand dissociates from the receptor. Some receptors, in spite of the continuing presence of the ligand, enter a closed conformational state known as the "desensitized" state. Desensitization is a characteristic property of many receptors in this superfamily that in addition to the GABA$_A$Rs includes the nicotinic acetylcholine receptors, the glycine receptors, and the ionotropic receptors for serotonin.

In the case of the GABA$_A$Rs, the five co-assembled subunits are different proteins. To date 19 different GABA$_A$R subunits have been identified, and these include α1–6, β1–4, γ1–3, δ, ε, θ, and ρ1–2 (Sieghart and Sperk, 2002; Whiting, 2003). Depending on the assembled subunits, GABA$_A$Rs have specific anatomical localization (Pirker et al., 2000) most likely as a result of various cell- or brain region-specific anchoring and trafficking mechanisms (Moss and Smart, 2001). Moreover, the physiological and pharmacological properties of the GABA$_A$Rs also depend on their subunit composition (Hevers and Lüddens, 1998; Mody and Pearce, 2004). The random combinations of the 19 subunits taken five-by-five and considering orientation specificity would result in quite a large number of different GABA$_A$R combinations. Yet, the total number of naturally occurring combinations is no more than a few dozen. This reduction in the degrees of freedom is made possible by limiting the subunit partners that can assemble together, and by imposing strict rules on the number of different subunits of the same class in a given assembly (Sieghart and Sperk, 2002; Whiting, 2003). Thus, the most prevalent combination of GABA$_A$Rs in the mammalian brain is that made of 2 α1, 2 β2, and 1 γ2 subunits arranged around the central pore in a particular order (the α1β2γ2 subunit combination). Specific GABA$_A$Rs assembled from different subunit combinations have different developmental, physiological, and pharmacological properties, and are also confined to specific compartments on a given cell (Hevers and Lüddens, 1998; Mody and Pearce, 2004). Therefore, these specific GABA$_A$Rs are of great interest for developing highly specific drug targets for the brain (Whiting, 2003).

The focus of this review is a subclass of GABA$_A$Rs that contain the δ subunit. The δ subunit was cloned many years ago, and was promptly shown to have a specific expression in the brain, specific pharmacological properties, most importantly lack of benzodiazepine sensitivity, and a mutual exclusion with γ subunits from receptor assemblies (Shivers et al., 1989). The preferred combination partners of δ subunits were the α6 and α4 subunits (from all the α's) and the β2 and β3 subunits (from all the β's). The δ subunits in combination with a6 subunits are mainly found in cerebellar granule cells, which constitute the highest density of δ subunits in the brain (Pirker et al., 2000). Outside of the cerebellum, the preferred partners of δ subunits are the α4 subunits. High densities of α4/δ subunit-containing GABA$_A$Rs are found in the thalamus, striatum, hippocampal dentate gyrus, olfactory bulb, and layers 2/3 of the neocortex. Several studies have confirmed using pharmacological approaches, null mutant mice or both that in the neurons where δ subunits are present, these GABA$_A$Rs are responsible for the mediation of tonic inhibition (Drasbek and Jensen, 2006; Glykys and Mody, 2006a; Glykys et al., 2007; Jia et al., 2005; Porcello et al., 2003; Stell et al., 2003; Wei et al., 2003).

The first indication about the peculiar subcellular localization of δ subunit-containing GABA$_A$Rs came from studies on cerebellar granule cells. In these neurons, δ subunit-containing GABA$_A$Rs is situated far from the synapses, scattered around the cell surface of the neurons (Nusser et al., 1998). In another area of the brain with high levels of δ subunits, in the granule cells of the dentate gyrus, these receptors are localized somewhat closer to the synapses, but still perisynaptically (Wei et al., 2003). This means that these receptors are ideally located to sense GABA overspilled after a synaptic release process from nearby boutons, or to be activated by the ambient levels of GABA present in the extracellular space. However, to function as receptors capable to detect low concentrations of extrasynaptic GABA, these receptors have to satisfy certain pharmacological criteria. First, they have to have a high affinity for GABA to be activated by the low concentration of transmitter in the extracellular space estimated to be in the range of one to a few μM (Kuntz et al., 2004; Nyitrai et al., 2006). Second, they have to be devoid of desensitization, as receptors in the continuing presence of agonist tend to desensitize and spend their time mainly in the closed configuration. Are these pharmacological properties met for the δ subunit-containing GABA$_A$Rs?

Several studies have shown the high affinity for GABA of the receptors containing δ subunits in combination with either α4 or α6 subunits. Their half maximal activation by GABA (EC$_{50}$) is in the tens of nM range, well within the range of GABA found in the extracellular space (Saxena and Macdonald, 1994; Wallner et al, 2003). The δ subunit-containing GABA$_A$Rs also have a low degree of desensitization in the continuous presence of agonist (Bianchi and Macdonald, 2003; Wohlfarth et al., 2002). This property is also essential for their role as mediators of a tonic ("always on") conductance. One of the other interesting pharmacological properties of the δ subunit-containing GABA$_A$Rs is that GABA is not a very efficacious agonist. This means that the coupling between binding of GABA and the opening of the channel is not the most effective one, in spite of the fact that very low concentrations of GABA can open the channels. Other agonists, such as THIP or (4,5,6,7-tetrahydroisoxazolo-[5,4-c]pyridine-3-ol, Gaboxadol) are more efficacious agonists than GABA itself at these receptors (Brown et al., 2002; Wafford and Ebert, 2006). Thus GABA is a high potency, but low efficacy agonist at the receptors mediating tonic inhibition in many central neurons. 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 is precisely what neurosteroids appear to be doing to the δ subunit-containing GABA_ARs (Bianchi and Macdonald, 2003; Wohlfarth et al., 2002). Neurosteroids (also called neuroactive steroids) are metabolites of ovarian steroids such as progesterone and of corticosteroids such as corticosterone (Belelli and Lambert, 2005). They can be synthesized in the brain by specific enzymes present in neurons and glial cells. The most potent positive endogenous modulators of GABA_A receptor function are the 3α-hydroxy ring A-reduced pregnane steroids that have sedative-hypnotic, anticonvulsant, and anxiolytic effects (Belelli and Lambert, 2005; Majewska et al., 1986). Neurosteroids in the nM concentration range, that is, the range assumed to be present in the extracellular space under various physiological and pathological conditions, selectively enhance the magnitude of tonic inhibition in cells in which this inhibition is mediated by δ subunit-containing GABA_ARs (Stell et al., 2003). The effect of neurosteroids on synaptic (phasic) inhibition does not occur until the neurosteroid reaches much higher concentrations than it is supposed to reach in the brain (Stell et al., 2003). The high potency of neurosteroids at δ subunit-containing GABA_ARs is interesting, as neurosteroids bind to α and β subunits (Hosie et al., 2006).

Ethanol sensitivity of GABA responses

The effects of alcohol on the human body and mind have been known for thousands of years. Late Stone Age beer jugs are proof for the existence of intentionally fermented beverages (around 10,000 B.C.), and it is possible that the consumption of beer may have preceded that of bread. Wine appears in Egyptian pictographs around 4,000 B.C. In spite of its long presence in human history, the mechanisms of action of ethanol on the brain are poorly understood. Although drinking and driving laws differ from country to country, in California the legal upper limit for blood alcohol level for operating a motor vehicle is 0.08%. This corresponds to approximately 17 mmol/l of ethanol in the blood. Commercial drivers are limited to a maximum of 0.04%, that is, around 8.5 mmol/l of ethanol in the blood. Because of various factors such as age, gender, weight, metabolic rate, etc., not every individual becomes impaired to the same extent by these particular ethanol concentrations, nevertheless, at least legally, these ethanol concentrations are considered to be the impairing the operation of motor vehicles. According to the California Alcohol and Drug Programs (www.adp.ca.gov) the average blood alcohol concentration (BAC) of a convicted DUI offender in 2003, as reported by law enforcement on adminstrative per se (APS) forms, was 0.161% (28 mM). Thus, for the purpose of this review we will consider ethanol concentrations in the range of 10–30 mM to be "sobriety impairing." Over the past 30 years several studies (Aguayo, 1990; Akk and Steinbach, 2003; Celentano et al., 1988; Davidoff, 1973; Kumar et al., 2004; Mereu and Gessa, 1985; Nestoros, 1980; Siggins et al., 1987; Suzdak et al., 1986; Ticku, 1989) have examined the effects of "low," that is, sobriety-impairing ethanol concentrations on various responses to GABA_AR activation in neurons, but no consensus has been reached, and no specific GABA_AR subunit combination could be identified as a specific ethanol target.

Recently, however, δ subunit-containing GABA_ARs expressed in *Xenopus laevis* oocytes have been shown to be uniquely sensitive to low ethanol concentrations (Sundstrom-Poromaa et al., 2002; Wallner et al., 2003). These findings have not remained unchallenged. Recent experiments in various expression systems including oocytes, the stable L(tk⁻) cell linescell line expressing δ subunit-containing GABA_ARs (Borghese et al., 2005) and CHO cells (Yamashita et al., 2006) ethanol failed to enhance currents evoked by GABA, but the reason for the discrepancies between the results in various expression systems remains unknown. The reader should consult recent review articles summarizing the findings in expression systems (Hanchar et al., 2004; Wallner et al., 2006), in genetically altered mice (Boehm et al., 2006), and in brain slice preparations (Weiner and Valenzuela, 2006) from which it is clear that certain GABA_AR combinations, including those containing δ subunits, may be responsible for specific actions of ethanol on the brain. In addition to the role of δ subunits, point mutations in the α1 subunit have also been shown to affect ethanol sensitivity, but only at higher (80 mM) ethanol concentrations (Werner et al., 2006). Moreover, enhancement of GABA release through presynaptic mechanisms has also been reported (Weiner and Valenzuela, 2006).

Ethanol sensitivity of tonic inhibition in neurons

The measurement of tonic inhibition

The term "tonic" in the case of inhibition is not meant to designate the carbonated beverage flavored with a small amount of quinine. By analogy to the tonic muscle contraction, tonic inhibition implies that the underlying conductance is "always on." Therefore, the presence of such a persistent conductance should be easily demonstrated if it generates a detectable amount of charge movement across the membrane by the use of a receptor antagonist. Indeed, the use of high resolution whole-cell recordings in neurons of brain slices and cell cultures allowed the recordings of persistent currents that could be blocked by the administration of GABA_AR antagonists (Bai et al., 2001; Brickley et al., 1996; Kaneda et al., 1995; Otis et al., 1991; Salin and Prince, 1996). The difference in the holding current between that recorded in the presence of the antagonist and that before antagonist administration is commonly referred to as the tonic current. New methods have been developed

based on all-point histograms that allow the simultaneous measurement of tonic and phasic conductances at desired time intervals (Glykys and Mody, 2006b), but the essential approach of using saturating antagonist concentrations to block both tonic and phasic conductances has remained the same. It is important to emphasize that saturating antagonist concentrations should be used to block 100% of both tonic and phasic inhibitions, because the relative affinities for GABA and for the antagonist of the different receptors mediating the two types of inhibition may differentially affect the two types of inhibitory activity (Stell and Mody, 2002).

Unfortunately, the different recording preparations and practices used by various laboratories make it difficult to come up with a consensus for the optimal recording conditions for tonic inhibition. As tonic inhibition is activated by the GABA levels present in the extracellular space, any factor that will control the presence of GABA in the extracellular space will have an impact on the magnitude of tonic inhibition. These include the activity of GABA uptake mechanisms, the level of spontaneous activity of GABAergic neurons, possible leakage of GABA from neurons or glial cells, the rate of perfusion of the slices, oxygenation levels, etc. It is also possible to record tonic current in the total absence of agonist-dependent activation of GABA$_A$Rs, when just the spontaneous openings of the channels are responsible for a steady conductance (Birnir et al., 2000; McCartney et al., 2006). Interestingly, the spontaneously opening receptors can be blocked by bicuculline but not by gabazine (McCartney et al., 2006) thus introducing an added cautionary note when using various antagonists to reveal the magnitude of tonic inhibition (Bai et al., 2001).

Ethanol sensitivity of the tonic inhibition mediated by δ subunit-containing GABA$_A$Rs

Tonic inhibition is present in various neurons in diverse brain regions. Thus far, various approaches including pharmacological and genetic knockout techniques identified several GABA$_A$R assemblies responsible for mediating tonic inhibition in the brain. Tonic inhibition can be mediated by GABA$_A$Rs containing δ subunits in combination with α6 subunits in cerebellar granule cells (Brickley et al., 2001; Stell et al., 2003), by partnering with α4 subunits in dentate gyrus granule cells (Chandra et al., 2006; Glykys et al., 2007; Stell et al., 2003), thalamic neurons (Chandra et al., 2006; Cope et al., 2005; Jia et al., 2005; Porcello et al., 2003), layer 2/3 cortical pyramidal cells (Drasbek and Jensen, 2006), and by pairing up with α1 subunits in interneurons of the dentate gyrus (Glykys et al., 2007). Other GABA$_A$R combinations without δ subunits known to mediate tonic inhibition in hippocampal pyramidal cells are those containing α5 subunits (Caraiscos et al., 2004; Glykys and Mody, 2006a) or simply αβ subunit combinations (Mortensen and Smart, 2006).

Only the tonic inhibition mediated by δ subunit-containing GABA$_A$Rs has been shown to be sensitive to ethanol in

the range of 20–30 mM (Glykys et al., 2007; Hanchar et al., 2005; Liang et al., 2006; Wei et al., 2004). Fig. 1 shows the sensitivity of the tonic current to 30 mM ethanol in ventro-basal (VB) thalamic neurons, where this type of inhibition is also mediated by δ subunit-containing GABA$_A$Rs (Chandra et al., 2006; Cope et al., 2005; Jia et al., 2005; Porcello et al., 2003). One report found no effect of 30 mM ethanol on the tonic current recorded in mouse dentate gyrus granule cells (Borghese et al., 2005). It has to be noted that in contrast to our recording conditions (Glykys et al., 2007; Wei et al., 2004) these authors used younger animals (P20-26, C57Bl/6 × 129SvJae mixed genetic background mice) and recorded a large tonic inhibitory current (mean = 19 pA) in the presence of TTX (Borghese et al., 2005), when this activity is significantly
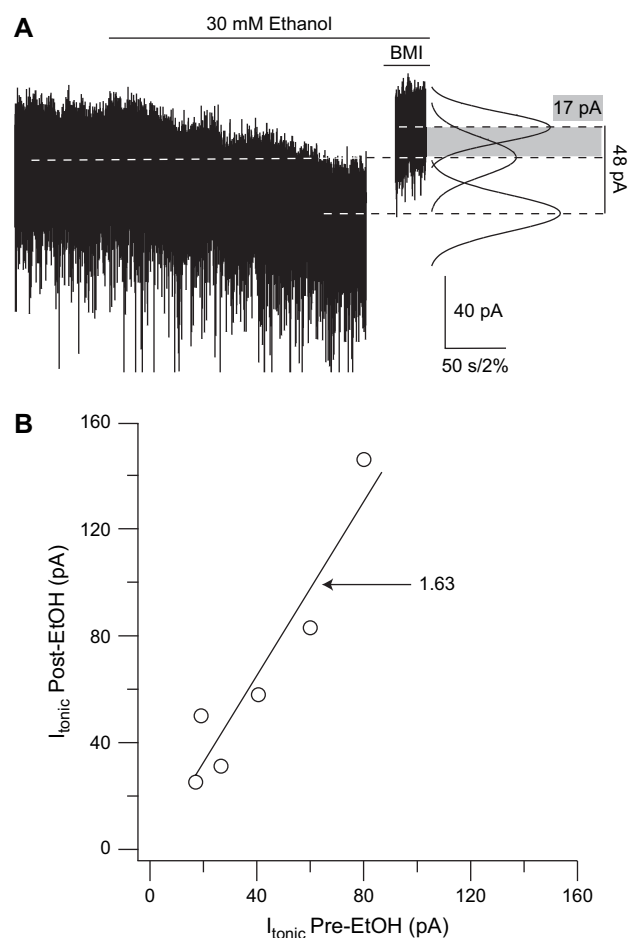
Fig. 1. Tonic inhibitory currents recorded from medium spiny thalamic neurons show potentiation by ethanol. (A) Voltage-clamp recording form a VB thalamic neuron (V_h = −70 mV). Tonic inhibitory current was enhanced by 30 mM ethanol (open bar). The magnitude of tonic inhibition was measured after the application of the gamma-aminobutyric acid receptor antagonist bicuculline methiodide (BMI; 100 μM). The dashed lines represent the mean holding current during the three conditions. The average current increased from 40.5 ± 10.2 to 65.5 ± 18.1 pA (*n* = 6). (B) Scatter plot of the tonic inhibitory currents before plotted against those after perfusion of 30 mM EtOH (Linear fit, slope 1.63 is significantly different *P* < .001 from a line with a slope of 1.0 that would indicate no effect, *R* = 0.95).

reduced by blocking action potentials in slices (Brickley et al., 1996; Glykys and Mody, 2006b; Kaneda et al., 1995). It is also curious that the size (mean = 72 pA) and the decay time constant (mean = 9.7 ms) of the miniature IPSCs (mIPSCs) recorded in the study (Borghese et al., 2005) are significantly larger than those reported by other investigators. It is also interesting to note that the tonic current recorded in cerebellar granule cells in culture does not respond to sobriety-impairing ethanol concentrations (Yamashita et al., 2006). The reason for the discrepancies remains to be determined, but it should be kept in mind that there are several factors that can directly influence the magnitude of tonic inhibition and ethanol may act on any of these to enhance it.

One of the most important regulators of tonic inhibition is the uptake of GABA (Jensen et al., 2003; Nusser and Mody, 2002; Semyanov et al., 2003; Wu et al., 2006). It is therefore conceivable that ethanol could alter tonic inhibition by decreasing the activity of the GABA transporter, thus elevating the levels of extracellular GABA. Fig. 2 shows an experiment in a dentate gyrus molecular layer interneuron that excludes this possibility. Ethanol enhanced the tonic current recorded in these neurons both in the absence and presence of NO711, a potent blocker of GABA uptake (Dalby, 2000). It is interesting to note that the potentiation by ethanol was similar regardless of the magnitude of the tonic current, which becomes quite large in the presence of the uptake blocker (Fig. 2). This is in contrast to the effects of benzodiazepines on tonic currents mediated by δ subunit-containing GABA$_A$Rs, which contrary to a well-established benzodiazepine-insensitive pharmacology, become potentiated by benzodiazepines when activated only by low GABA concentrations (Santhakumar et al., 2006). A point mutation in position 100 (Q/R) of the α6 subunit markedly affects benzodiazepine and ethanol sensitivities (Hanchar et al., 2005; Santhakumar et al., 2006). Therefore, we also wanted to know whether the H101R mutation that abolishes benzodiazepine sensitivity in α1 subunit-containing GABA$_A$Rs (Wieland et al., 1992) results in a change of their modulation by ethanol. In α1^{H101R} knock-in mice (Rudolph et al., 1999), a His residue in position 101 in α1 subunits is changed to an Arg as in α4 (Fig. 3), thus abolishing benzodiazepine sensitivity. Recordings in molecular layer interneurons, where the tonic current is mediated by α1βxδ GABA$_A$R combinations (Glykys et al., 2007), in knock-in mice with this amino acid substitution showed a tonic inhibition similar to that in wild-type (WT), and a comparable potentiation by 30 mM ethanol (Control 16.9 ± 1.9 vs. EtOH 26.9 ± 2.8 pA; $P = .0005$ paired t-test; $n = 14$; Fig. 3). The potentiation ratio was not different from WT (WT 2.05 ± 0.3 vs. α1^{H101R} 1.74 ± 0.2; $P = .332$ unpaired t-test assuming unequal variances; $n = 10$ and 14, respectively) suggesting that the α1^{H101R} mutation that renders α1 subunits at a residue critical for benzodiazepine sensitivity to be more like α4 subunits, is not a factor for the potentiating effects of ethanol.
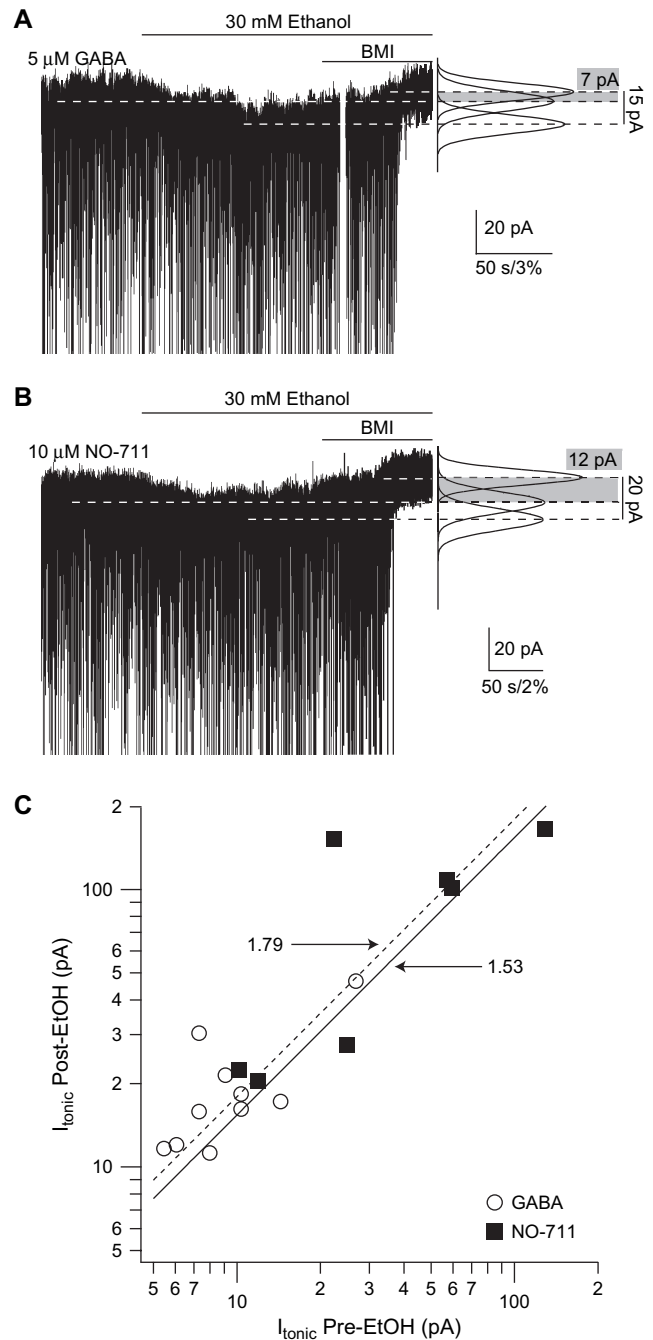

Fig. 2. Tonic inhibition enhancement by ethanol is present when gamma-aminobutyric acid (GABA) is added to the aCSF as well as when GABA transporters are blocked. (A) Voltage-clamp recordings from dentate gyrus molecular layer interneurons showing the ethanol-induced potentiation of the tonic inhibitory current in the presence of 5 µM GABA. ($V_h = −70$ mV, horizontal dashed lines indicate perfusion of ethanol and BMI); Right, Gaussian fits to the all-point histograms of the baseline current during each condition. (B) Same as above but in the presence of 10 µM NO-711. (C) Scatter plot of the tonic inhibitory currents before plotted against those after perfusion of 30 mM EtOH (Linear fit: GABA, [dashed line] slope 1.79 $R = 0.81$; NO-711 [solid line] slope 1.53, $R = 0.72$; both slopes are significantly different $P < .001$ from line with a slope of 1.0 that would indicate no effect of ethanol). Note the logarithmic scale that was necessary to use for the very large tonic inhibitory currents in the presence of NO-711.
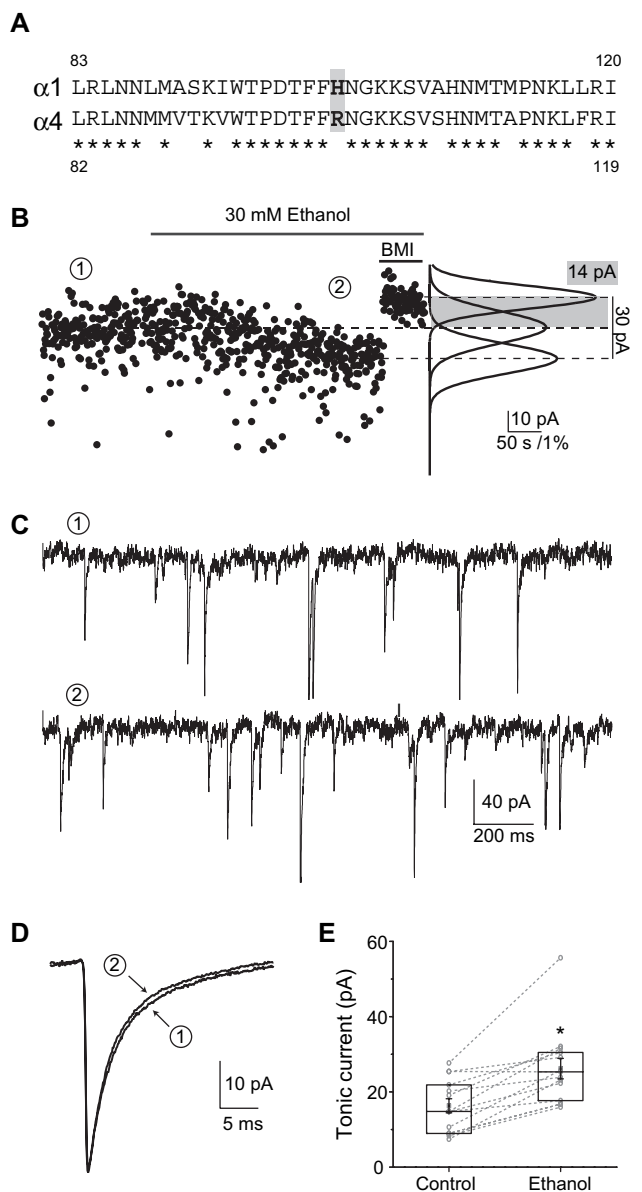
Fig. 3. Tonic inhibition and its potentiation by ethanol are unaltered in α1[H101R] dentate gyrus molecular layer interneurons. (A) Amino acid alignment between α1 and α4 gamma-aminobutyric acid receptor subunits showing the position of the critical amino acid residue difference for benzodiazepine BZ sensitivity. Asterisks indicate identical amino acids. (B) Voltage-clamp recordings from α1[H101R] interneurons show a potentiation of the tonic inhibitory current by 30 mM ethanol. Left, baseline current plotted at 500-ms intervals in control (①), 30 mM ethanol (②), and BMI conditions ($V_h = -70$ mV, horizontal bars indicate perfusion of the drugs); Right, Gaussian fits to the all-point histograms of the baseline current recorded during each condition. (C) Recording segments low pass filtered at 1 KHz in control and ethanol conditions. (D) Averaged spontaneous inhibitory postsynaptic current (sIPSC) recorded in control and ethanol conditions from two different cells. (E) Box chart of all tonic currents recorded. Box represents the 25, 50, and 75 percentiles, with the superimposed mean ± S.E.M. Circles connected by lines represent paired individual values of the tonic inhibitory currents recorded in a given cell under the two conditions. Asterisk represents $P = .0005$ paired t-test; $n = 14$.

From the data presented above, it is evident that tonic inhibition mediated by δ subunit-containing GABA$_A$Rs is an important target for sobriety-impairing ethanol concentrations in the brain. It remains to be determined whether various intra- or extracellular factors modulate the action of ethanol on this type of tonic inhibition. Furthermore, it is presently unclear if the receptors underlying tonic inhibition also play a role in alcohol addiction and tolerance.

Tonic inhibition as a common target for ethanol and neurosteroids

We recently demonstrated dynamic, ovarian cycle-linked modifications in specific GABA$_A$R expression and function (Maguire et al., 2005). During the stage of the estrous cycle in mice when levels of progesterone and of progesterone derivatives locally synthesized in the brain (neurosteroids) are elevated, there is an increased expression of the GABA$_A$R δ subunits in the membranes of hippocampal neurons and an increase in GABA$_A$R δ subunit-mediated tonic inhibition in dentate gyrus granule cells. This increase in GABA$_A$R δ subunits corresponds to a period of lowered seizure susceptibility and anxiety (Maguire et al., 2005). Other investigators have shown the same receptors to parallel ovarian cycle-related changes in the periaqueductal grey matter (Griffiths and Lovick, 2005; Lovick, 2006; Lovick et al., 2005) or to be upregulated in a steroid withdrawal model of premenstrual dysphoric disorder (PMDD) (Smith et al., 2006). The changes in the GABA$_A$R δ subunits during the ovarian cycle and the associated alterations in neuronal excitability and anxiety may be highly relevant to the common psychiatric and neurological disorders such as PMDD, its milder form premenstrual syndrome (PMS), and postpartum depression that affect women during fluctuating changes in ovarian steroid levels as take place during the menstrual cycle and pregnancy.

Consistent with the common involvement of the δ subunit-containing GABA$_A$Rs in ovarian cycle-related anxiety and in mediating the effects of ethanol, women with PMS have increased their alcohol consumption during the luteal phase (Charette et al., 1990; McLeod et al., 1994; Perry et al., 2004; Tobin et al., 1994), which may be an indication of self-medication since, as shown above, the function of δ subunit-containing GABA$_A$Rs and the tonic inhibition mediated by these receptors are enhanced by sobriety-impairing concentrations of ethanol.

A recent report highlighted that effect of the neurosteroid allopregnanolone on prolonging the long open times of single α1β2γ2L GABA$_A$R channels expressed in human embryonic kidney (HEK 293) cells activated by 50 μM GABA was shifted to 100-fold lower neurosteroid concentrations in the presence of 17 mM ethanol (Akk et al., 2007). However, this effect was not evident in the whole-cell currents evoked by GABA, possibly because of the relatively small contribution of the long openings to the total

current. Nevertheless, these findings illustrate that the δ sub-unit-containing GABA$_A$Rs may not be the only receptor subtype to mediate interactions between neurosteroid and ethanol effects.

Summary

The δ subunit-containing GABA$_A$Rs and consequently the tonic inhibition mediated by these receptors appears to be an important target for sobriety-impairing concentrations of ethanol. Stress- and ovarian steroid-derived neurosteroids also appear to act primarily on this type of inhibition, thus putting tonic inhibition mediated by δ sub-unit-containing GABA$_A$Rs in the crosshairs for finding effective therapies against a large number of psychiatric and neurological disorders related to alcoholism, stress, ovarian cycle, and pregnancy.

Acknowledgments

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