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Ethanol-enhanced GABA release: A focus on G protein-coupled receptors

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Abstract

While research on the actions of ethanol at the GABAergic synapse has focused on postsynaptic mechanisms, recent data have demonstrated that ethanol also facilitates GABA release from presynaptic terminals in many, but not all, brain regions. The ability of ethanol to increase GABA release can be regulated by different G protein-coupled receptors (GPCRs), such as the cannabinoid-1 receptor, corticotropin-releasing factor 1 receptor, $GABA_B$ receptor, and the 5-hydroxytryptamine 2C receptor. The intracellular messengers linked to these GPCRs, including the calcium that is released from internal stores, also play a role in ethanol-enhanced GABA release. Hypotheses are proposed to explain how ethanol interacts with the GPCR pathways to increase GABA release and how this interaction contributes to the brain region specificity of ethanol-enhanced GABA release. Defining the mechanism of ethanol-facilitated GABA release will further our understanding of the GABAergic profile of ethanol and increase our knowledge of how GABAergic neurotransmission may contribute to the intoxicating effects of alcohol and to alcohol dependence.

Keywords

Ethanol; Presynaptic; GABA release; G protein-coupled receptor; Internal calcium store

1. Introduction

While previously thought to be just an amino acid, gamma-aminobutyric acid (GABA) was recognized as a major inhibitory neurotransmitter in the central nervous system during the 1960s (Krnjevic and Phillis, 1963; Otsuka et al., 1966; Roberts and Kuriyama, 1968). In the presynaptic terminal, glutamate decar-boxylase converts glutamate into GABA, and GABA is actively transported into the synaptic vesicles by a vacuolar proton pump that establishes an electrochemical gradient across the vesicle (Martin, 1993; McIntire et al., 1997; Sagne et al., 1997). The vesicles dock at the plasma membrane through an interaction between

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synaptobrevin, which is located on the synaptic vesicles, and syntaxin 1 and SNAP-25, which are located on the plasma membrane (see Sudhof, 1995, 2004). This core complex allows for a number of protein–protein interactions to occur that are necessary for exocytosis, including the calcium sensor synaptotagmin triggering the final stage of vesicle fusion with the membrane. Once the vesicles fuse with the membrane, GABA is released from the presynaptic terminal into the synaptic cleft and binds to the postsynaptic $GABA_A$ receptors, allowing chloride to flow into the neuron.

The flow of chloride through the $GABA_A$ receptor can be measured in an *in vitro* slice preparation using whole-cell voltage-clamp recordings. This technique can measure both spontaneous (action potential-independent and dependent) and evoked GABA release. Action potential-independent GABA release is isolated by tetrodotoxin—a drug that blocks action potentials via inhibition of voltage-gated sodium channels. Treatment with tetrodotoxin allows for measurement of a $GABA_A$ receptor-mediated chloride flux response known as a miniature inhibitory postsynaptic current (mIPSC). When the number of mIPSC events is increased, the change is interpreted as an increase in spontaneous GABA release. In the absence of tetrodotoxin, there is a mix of action potential-dependent and independent GABA release, and this measured $GABA_A$ receptor-mediated chloride flux response is referred to as a spontaneous inhibitory postsynaptic current (sIPSC). Interpreting an increase in sIPSC frequency is not as straightforward as interpreting an increase in mIPSC frequency because an increase in sIPSC frequency can be due to a direct effect on the presynaptic terminal (i.e., increase in terminal release probability), an increase in action potential firing, or activation of a neighboring neuron that affects the presynaptic neuron (i.e., increase in somatic/axonal excitability).

In contrast to spontaneous GABA release, measuring evoked GABA release involves stimulating an action potential to induce GABA release. This measured $GABA_A$ receptor-mediated chloride flux response is known as an evoked inhibitory postsynaptic current (eIPSC). Because there is no direct readout for changes in presynaptic release with analysis of eIPSCs, a paired pulse ratio (PPR) is used to make this assessment. The PPR is the ratio of the amplitudes of two eIPSCs ($eIPSC_2/eIPSC_1$) that are evoked by stimuli applied 20–200 ms apart (for review see Zucker, 1989). A change in the PPR is inversely related to a change in evoked neurotransmitter release (Siggins et al., 2005); therefore, if ethanol decreases the PPR, it is interpreted as ethanol increasing evoked GABA release. Through the use of whole-cell voltage-clamp recordings and the analysis of mISPCs, sIPSCs, and eIPSCs, it was determined that ethanol increases GABA release in the brain slice preparation, which will be discussed in greater detail below.

2. The history of ethanol-enhanced GABA release

Starting in the late 1970s, there were a number of behavioral studies showing an interaction between ethanol and drugs that affect the $GABA_A$ receptor (for reviews see Criswell and Breese, 2005; Siggins et al., 2005; Weiner and Valenzuela, 2006), which led the field to focus on ethanol–GABA interactions. One of the earliest studies on the effect of ethanol on GABA function interpreted an overall decrease in brain GABA content following a 4.3 g/kg ethanol dose as an indication that ethanol releases GABA from neurons (Gordon, 1967). However, several subsequent studies failed to reproduce this effect (Frye and Breese, 1982; Sutton and Simmonds, 1973; Volicer and Klosowicz, 1979). With the development of the dialysis probe, additional studies produced varied but primarily negative results. Systemically administering ethanol did not change GABA dialysate levels in the anterior cingulate cortex (Zuo et al., 2007), hippocampus (Dahchour and De Witte, 1999; Ward et al., 2009), ventral tegmental area (VTA; Kemppainen et al., 2010), or nucleus accumbens (Smith et al., 2004). Systemic ethanol administration decreases GABA dialysate levels in the

cerebellar nuclei (Manto et al., 2005) and ventral pallidum (Kemppainen et al., 2010). There was also an ethanol-induced decrease in GABA dialysate in the nucleus accumbens in alcohol-tolerant, but not alcohol-nontolerant, rat lines (Piepponen et al., 2002) and in alcohol-dependent rats (Dahchour and De Witte, 2000). Thus, the preponderance of dialysis data indicated a lack of ethanol effect on GABA release, or even a slight decrease, following systemic ethanol administration.

When intracellular recordings were first used to study the GABAergic effect of ethanol, Carlen et al. (1982) found that ethanol enhanced the size of eIPSCs recorded from the CA1 region of the hippocampus. While analyzing eIPSC size does not indicate whether ethanol is acting pre- or postsynaptically (see Introduction), Carlen et al. (1982) nonetheless postulated that the increase in eIPSC size was due to a presynaptic mechanism. Based upon a later discovery that inhibition of $GABA_B$ receptors is necessary for ethanol to consistently increase eIPSC amplitude in the hippocampus (Wan et al., 1996), the authors speculated that these $GABA_B$ receptors were located presynaptically (Siggins et al., 1999). An experiment with spinal cord neurons found that ethanol increases sIPSC frequency without having an effect on sIPSC amplitude (Cheng et al., 1999). Together, these data provided the first clues that ethanol might act at the presynaptic terminal to increase the release of GABA.

In 2002 Dr. Dennis Twombly organized a National Institute on Alcohol Abuse and Alcoholism workshop to discuss the deficiency in studies exploring the presynaptic effects of ethanol (see Roberto et al., 2006). By determining if ethanol had an effect on mIPSC frequency and the PPR, a number of reports thereafter provided support for ethanol increasing spontaneous and evoked GABA release, respectively, in a number of brain regions (see Table 1 for summary). One of the first studies showed that ethanol increases both spontaneous and evoked GABA release in the central nucleus of the amygdala (CeA; Roberto et al., 2003). In addition to the CeA (Nie et al., 2004; Roberto et al., 2003), ethanol has been shown to increase GABA release in the following brain regions: basolateral amygdala (Silberman et al., 2008; Zhu and Lovinger, 2006), hippocampus (Ariwodola and Weiner, 2004; Li et al., 2006; Sanna et al., 2004), VTA (Melis et al., 2002; Theile et al., 2008), cerebellum (Carta et al., 2004; Criswell et al., 2008; Hirono et al., 2009; Kelm et al., 2007; Kelm et al., 2008; Ming et al., 2006), substantia nigra (Criswell et al., 2008) and brainstem/spinal cord (Sebe et al., 2003; Ziskind-Conhaim et al., 2003). Fig. 1 includes data collected from the cerebellar interneuron-Purkinje cell synapse demonstrating that ethanol increases mIPSC frequency (Fig. 1A and B) by increasing vesicular release of GABA (Fig. 1C) while having little or no effect postsynaptically (Fig. 1D). While ethanol has been shown to increase GABA release in a number of brain regions with the use of slice electrophysiology, ethanol does not increase GABA release in every brain region; specifically, ethanol has no effect on GABA release in the cortex, lateral septum, and thalamus (Criswell et al., 2008; Jia et al., 2008). In the brain regions where ethanol does increase GABA release, significant progress has been made regarding the underlying mechanism responsible for this action of ethanol.

The dichotomy between the effects of locally applied ethanol, which usually increases GABA release when measured with intracellular recordings, and systemic ethanol, which usually has no effect on GABA release when measured with dialysis, suggests that the net effect of ethanol on intact neural circuits may differ from the effect of ethanol on individual neurons. This difference may be due to ethanol increasing GABA release onto inhibitory interneurons and thereby decreasing the subsequent release of GABA from the interneurons (see Celada et al., 1999; Tan et al., 2010). Thus, local application of ethanol may be necessary to study the immediate mechanism of ethanol-enhanced GABA release while the indirect effect of ethanol on GABA function may be modeled with dialysis experiments. Interestingly, when ethanol was applied locally by dialysis into the CeA, ethanol increased

GABA release, which verified the effect of bath-applied ethanol on CeA neurons in the slice preparation (Roberto et al., 2004, 2010).

3. G protein-coupled receptors influence ethanol-enhanced GABA release

A number of laboratories have demonstrated that the ability of ethanol to increase GABA release can be regulated by different G protein-coupled receptors (GPCRs, see Table 2 for summary). For example, the functionality of a $G\alpha_s$- or $G\alpha_q$-coupled GPCR is necessary for ethanol to increase GABA release in certain brain regions. Inhibition of the corticotropin-releasing factor (CRF)1 receptor, a GPCR coupled to $G\alpha_s$, blocks ethanol from increasing GABA release in the CeA (Nie et al., 2004;Roberto et al., 2010). This finding suggests that activation of the CRF1 receptor is essential for ethanol to increase the release of GABA in this brain region. Inhibition of the $G\alpha_q$-coupled 5-hydroxytryptamine-2C ($5\text{-}HT_{2C}$) receptor blocks ethanol-enhanced GABA release in the VTA, which suggests that activation of the $5\text{-}HT_{2C}$ receptor is essential for ethanol to increase GABA release at this site (Theile et al., 2009). More studies should be conducted to determine if activation of $G\alpha_s$- or $G\alpha_q$-coupled GPCRs is necessary for ethanol to induce GABA release in different brain regions.

Agonists for $G\alpha_i$-coupled GPCRs have the same inhibitory effect on ethanol-enhanced GABA release as *antagonists* for the $G\alpha_s$/$G\alpha_q$-coupled GPCRs. In the basolateral amygdala, activation of the $G\alpha_i$-coupled cannabinoid-1 (CB1) receptor (Talani and Lovinger, 2008) or activation of the $G\alpha_i$-linked $GABA_B$ receptor (Silberman et al., 2009; Zhu and Lovinger, 2006) inhibits ethanol-enhanced GABA release. At the cerebellar interneuron-Purkinje cell synapse, the ability of ethanol to increase GABA release is likewise blocked by activation of CB1 receptors or $GABA_B$ receptors (Kelm et al., 2007, 2008). In the CeA, activation of either the nociceptin/orphanin FQ peptide receptor (Roberto and Siggins, 2006), a $G\alpha_i$-coupled GPCR, or the CB1 receptor (Roberto et al., 2008) blocks ethanol from enhancing GABA release. Consistent with *activation* of $G\alpha_i$-coupled GPCRs *inhibiting* ethanol-enhanced GABA release, *inhibition* of $G\alpha_i$-coupled GPCRs *enhances* the ability of ethanol to increase GABA release. In the basolateral amygdala (Ariwodola and Weiner, 2004; Silberman et al., 2009) and hippocampus (Zhu and Lovinger, 2006), an antagonist for $G\alpha_i$-linked $GABA_B$ receptors enhances the ability of ethanol to increase GABA release, as does an antagonist for the $G\alpha_i$-linked CB1 receptors in the basolateral amygdala (Talani and Lovinger, 2008). In the CeA, an antagonist at the $G\alpha_i$-linked δ-opioid receptor augments the ability of ethanol to increase the release of GABA (Kang-Park et al., 2007).

Interestingly, the presence of a $G\alpha_i$-coupled GPCR does not guarantee that activation or inhibition of the receptor will affect ethanol-enhanced GABA release. Both μ-opioid receptor null mice and a μ-opioid receptor antagonist increase baseline GABA release in the CeA but have no effect on the ability of ethanol to increase GABA release in the CeA (Kang-Park et al., 2009). A $GABA_B$ receptor antagonist increases baseline GABA release in the cerebellum, but inhibition of $GABA_B$ receptors does not affect ethanol-enhanced GABA release in this brain region (Kelm et al., 2008). In the VTA neither a $GABA_B$ receptor agonist nor antagonist had an effect on the ability of ethanol to increase GABA release, despite the fact that both the agonist and antagonist affected baseline release of GABA (Theile et al., 2008). Given that agonists and antagonists for the GPCRs mentioned above affect baseline spontaneous GABA release, it is likely that these GPCRs are expressed at the presynaptic terminals. Because the presence of a functional GPCR at a presynaptic terminal does not guarantee that an agonist/antagonist for that GPCR will affect ethanol-enhanced GABA release, it is unlikely that ethanol acts directly on a GPCR to regulate release of GABA.

Instead of binding directly to a GPCR to influence GABA release, ethanol could act upstream of the GPCR to change the amount of ligand reaching the GPCR, or ethanol could indirectly alter the affinity of the GPCR for its ligand or the constitutive activity of the GPCR. The appropriate endogenous ligands are present in the CeA and the VTA, which are sites where activation of the CRF1 receptor (Nie et al., 2004) and 5-HT_{2C} receptor (Theile et al., 2009), respectively, is required for ethanol-enhanced GABA release. Specifically, GABAergic interneurons synthesize CRF in the CeA (Veinante et al., 1997), and serotonergic afferents innervate neurons in the VTA (Herve et al., 1987). However, ethanol could also act downstream from a GPCR to increase GABA release by binding to adenylate cyclase (Yoshimura et al., 2006) or protein kinase Cε (Das et al., 2009), and these possibilities have not been tested.

To test whether ethanol is acting upstream or downstream of the 5-HT_{2C} receptor to induce GABA release in the VTA, an illuminating experiment would be to block synthesis of 5-HT with parachlorophenylalanine (PCPA) and determine if ethanol can still increase GABA release. Because activation of the 5-HT_{2C} receptor is required for ethanol to increase GABA release in the VTA (Theile et al., 2009), a lack of effect of ethanol in the presence of PCPA would indicate that endogenous 5-HT is essential for this ethanol action. If PCPA blocks ethanol-enhanced GABA release, administration of a 5-HT_{2C} agonist (Ro-60-0175) to the slice in the absence of ethanol in the 5-HT-depleted tissue would be expected to increase GABA release. If ethanol does not induce a further increase in GABA release in the presence of the 5-HT_{2C} agonist, this outcome would suggest that ethanol influences levels of serotonin to increase GABA release. On the other hand, if ethanol induces a further increase in GABA release in the presence of both PCPA and the 5-HT_{2C} receptor agonist, this outcome would suggest that ethanol can act downstream of the GPCR. Described next is evidence that downstream GPCR-linked second messenger pathways are also involved in the ability of ethanol to increase GABA release.

4. Evidence that GPCR-linked intracellular messengers influence ethanol-enhanced GABA release

Activation of $G\alpha_s$-, $G\alpha_q$-, and $G\alpha_i$-coupled GPCRs affects signaling through different intracellular messenger pathways (see Fig. 2). Both the $G\alpha_i$ and $G\alpha_s$ subunits modulate adenylate cyclase with $G\alpha_s$ stimulating adenylate cyclase and $G\alpha_i$ inhibiting it. When adenylate cyclase is activated, it converts adenosine-5′-triphosphate (ATP) into 3′-5′-cyclic adenosine monophosphate (cAMP), which can bind to protein kinase A (PKA) regulatory subunits (Hanoune and Defer, 2001). The binding of cAMP to PKA frees the PKA catalytic subunits from the regulatory subunits, allowing the catalytic subunits to phosphorylate nearby targets. The $G\alpha_q$ subunit activates phospholipase C (PLC), which catalyzes the conversion of phosphoinositol 4,5-bisphosphate into diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP_3;Kiselyov et al., 2003). The released calcium from activation of the IP_3R and the presence of DAG both activate conventional protein kinase C (PKC) isoforms (α, β_I, β_{II}, γ). Given the involvement of GPCRs in ethanol-enhanced GABA release, it seems logical that the intracellular messengers linked to the GPCRs would influence the ethanol-induced increase in GABA release.

In the presence of adenylate cyclase antagonists (dideoxya-denosine and SQ 22,536) or PKA antagonists (H-89 and Rp-cAMP), ethanol was not able to significantly increase GABA release at the interneuron-Purkinje cell synapse (Hirono et al., 2009; Kelm et al., 2008). Additionally, a PLC antagonist (edelfosine) and two general PKC antagonists (chelerythrine and calphostin C) significantly blocked ethanol from increasing spontaneous GABA release at the interneuron-Purkinje cell synapse (Kelm et al., 2010). Studies with a PKCε antagonist and PKCε null mice found that PKCε is required for ethanol to increase GABA release in

the CeA (Bajo et al., 2008). Importantly, Bajo et al. (2008) also found that PKCε is required for CRF to increase GABA release through activation of the CRF1 receptor, but it is unknown if PKCε is facilitating activation of the CRF1 receptor or if activation of the CRF1 receptor leads to activation of PKCε. Overall, these results suggest that the $G\alpha_q$- and $G\alpha_s$-coupled GPCR pathways play a critical role in the ability of ethanol to increase GABA release.

While the $G\alpha_q$-coupled (i.e. PLC/PKC) and $G\alpha_s$-coupled (i.e. adenylate cyclase/PKA) pathways are both necessary for ethanol to increase spontaneous GABA release at the interneu-ron-Purkinje cell synapse, it is unknown if ethanol activates each pathway separately or if there is cross-talk occurring between the pathways. Cross-talk occurs between PKA and PKC at the GABAergic nucleus basalis of Meynert synapse (Kubota et al., 2003), and there are effects of ethanol that involve cross-talk between these protein kinases. For example, ethanol increases adenylate cyclase isoform 7 activity through a PKCδ-mediated mechanism, which leads to activation of PKA (Tabakoff et al., 2001). Ethanol also induces PKCε translocation to the cytosol through a PKA-dependent mechanism (Yao et al., 2008); specifically, this translocation of PKCε is thought to involve PKA activation of PLCβ. If ethanol is activating the pathways separately, there could be no convergence of the pathways, and PKA and PKC could act directly at the release machinery (Boczan et al., 2004; Lou et al., 2008). However, the pathways could converge at the internal calcium stores (see Fig. 2) because phosphorylation of the IP_3R and ryanodine receptor (RyR) by PKA and PKC increases the amount of calcium release from the internal stores (Bardo et al., 2006; Bugrim, 1999; Mignery et al., 1990; Patterson et al., 2004; Sobie et al., 2006). As discussed below, calcium release from internal stores plays an important role in ethanol-enhanced GABA release.

5. The role of calcium in ethanol-enhanced GABA release

It is well established that changes in presynaptic intracellular calcium levels can alter spontaneous and evoked GABA release (Bardo et al., 2002, 2006; Glitsch, 2008; Yamasaki et al., 2006). Incubating a cerebellar slice with a calcium chelator blocks the ability of ethanol to increase GABA release at the interneuron-Purkinje cell synapse (Kelm et al., 2010). Because limiting exposure of a membrane impermeable calcium chelator to the postsynaptic neuron had no effect on this ethanol mechanism (Kelm et al., 2007), these results suggest that a presynaptic, calcium-dependent mechanism is mediating ethanol-enhanced GABA release at this synapse. To investigate the role of extracellular calcium in this ethanol mechanism, a mechanically dissociated neuron preparation was used instead of a slice to allow for instantaneous access of the calcium-free extracellular solution to the synapse. This approach reduces the likelihood of simultaneously decreasing both intracellular and extracellular calcium levels. Ethanol was still able to increase spontaneous GABA release at the mechanically dissociated Purkinje cell synapse in a calcium-free extracellular solution or in the presence of a voltage-dependent calcium channel inhibitor (Kelm et al., 2007). These results demonstrate that, while calcium signaling does play a role in this ethanol mechanism, this calcium is not coming from an extracellular source, a finding which suggests that this calcium signaling is occurring intracellularly.

One potential source of intracellular calcium is the internal calcium store, which allows for increases in calcium release through activation of IP_3Rs and RyRs. Therefore, it is likely that IP_3Rs play a central role in ethanol-enhanced GABA release because an IP_3R antagonist blocks ethanol-enhanced GABA release in the cerebellum (Kelm et al., 2007) and VTA (Theile et al., 2009). Similarly, blocking calcium release from the RyRs also inhibits ethanol-enhanced GABA release (Kelm et al., 2007). After the internal stores are depleted of calcium, a *sarco* (endo)plasmic reticulum Ca^{2+} ATPase (SERCA) pump senses this

depletion and replenishes the stores (Kiselyov et al., 2003). After using a SERCA pump antagonist in a protocol that depletes the internal stores of calcium, the ability of ethanol to increase GABA release is blocked in the cerebellum (Kelm et al., 2007) and VTA (Theile et al., 2009). Theile et al. (2009) also found that the ability of a 5-HT_{2C} receptor agonist to increase GABA release in the VTA is blocked by a SERCA pump antagonist. This finding is consistent with 5-HT_{2C} receptors activating a $G\alpha_q$-protein that results in a subsequent release of calcium through activation of the IP_3Rs (Kiselyov et al., 2003), a process which supports the internal calcium stores being necessary for a 5-HT_{2C} receptor agonist to increase release of GABA. As noted earlier, the ability of ethanol to increase GABA release in the VTA is dependent on activation of the 5-HT_{2C} receptor (Theile et al., 2009). Overall, these data suggest that calcium release from internal stores plays an important role in ethanol-enhanced GABA release.

6. The brain region-specificity of ethanol-enhanced GABA release

As noted above, while ethanol enhances GABA release in several brain regions, it is ineffective at increasing GABA release in the cortex, lateral septum and thalamus (Criswell et al., 2008; Jia et al., 2008). However, the mechanism mediating this brain region-specificity is unknown. One explanation for the lack of ethanol effect could be the absence of the relevant GPCRs at these presynaptic terminals. If ethanol acts upstream of the GPCRs to release ligand, ethanol may not be able to increase the ligand concentration near the appropriate GPCRs. Further, other factors could act indirectly to decrease the affinity or constitutive activity of the GPCR. Alternatively, if both a $G\alpha_i$- and $G\alpha_s$-coupled GPCR are activated by ethanol on the same terminal, the effect of one GPCR could negate the effect of the other GPCR, leading to an overall lack of effect of ethanol on GABA release.

The regional specificity of ethanol-enhanced GABA release could also be mediated downstream from the GPCRs, with a likely source being the internal calcium stores. Blockade of RyRs or IP_3Rs or depletion of internal calcium stores prevents ethanol-enhanced GABA release while leaving a large portion of spontaneous GABA release intact (Kelm et al., 2007; Theile et al., 2009). Thus, ethanol appears selective for the subtype of GABA release that is dependent on calcium release from internal stores. If the proportion of GABA release dependent upon internal calcium stores differs between brain regions, as has been suggested (Yamasaki et al., 2006), brain regions with a low dependence on internal stores would be less sensitive to the effect of ethanol on GABA release than regions with a high dependence. Finally, the regional differences could be due to a differential function of one or more of the multiple intracellular messenger pathways involved in ethanol-enhanced GABA release.

Therefore, much work is needed to understand this regionally specific effect of ethanol on GABA release. While it is possible that this lack of ethanol effect on GABA release in certain brain regions could be due to an artifact introduced by the techniques used to prepare the tissue or record from the neurons, this possibility seems unlikely because Criswell et al. (2008) used the same preparation to show that ethanol increases GABA release in one brain region while having no effect in another. Defining the basis of this regional specificity of ethanol should be a priority because this new information will likely facilitate a broader understanding of the means by which ethanol alters brain function via GABAergic mechanisms.

7. Functional relevance of ethanol-enhanced GABA release

While there is clear evidence that ethanol increases GABA release, the behavioral and functional relevance of this ethanol-induced increase in GABA release is less clear. To date, all of the manipulations that influence the ability of ethanol to release GABA have other

effects that are independent from ethanol-enhanced GABA release; therefore, we cannot directly link any behavioral consequences to ethanol-induced GABA release. For example, a CRF1 receptor antagonist prevents ethanol-enhanced GABA release in the CeA (Nie et al., 2004) and also blocks the increased anxiety associated with repeated ethanol withdrawals (Huang et al., 2010; Overstreet et al., 2004; Wills et al., 2009). While the correlation between the prevention of ethanol-enhanced GABA release and the decrease in withdrawal-induced anxiety is solid, the causal link is unclear. Furthermore, ethanol concentrations below 20 mM have been ineffective at enhancing GABA release (Zhu and Lovinger, 2006). A 20 mM ethanol concentration is equivalent to a blood ethanol level of almost 0.1%, which is a concentration that is seldom reached by social drinkers, suggesting that many of the effects of mild intoxication are not mediated by ethanol increasing GABA release.

Because binge drinkers often exceed a 20-mM ethanol concentration, one possibility is that this deleterious drinking pattern selectively initiates ethanol-induced GABA release to contribute to the long-term consequences of alcohol abuse. Further, chronic ethanol intake does not appear to result in tolerance to the effect of ethanol on GABA release (Roberto et al., 2004, 2006). Thus, the increased ethanol intake required to produce the same inebriating effect in a tolerant individual would result in an increase in any deleterious effects associated with the elevated release of GABA. In ethanol-dependent rats who are in withdrawal, there is an increase in GABA dialysate and mIPSC frequency in the CeA (Roberto et al., 2004), and administration of a CRF1 receptor antagonist blocks this increase in GABA release (Roberto et al., 2010). This study suggests that there is a homeostatic effect of GABA release during withdrawal from chronic ethanol, and that the relationship between acute ethanol exposure and the GPCRs in the mechanism of ethanol-enhanced GABA release could extend to chronic ethanol. Therefore, while more work is needed to determine the behavioral relevance of ethanol-enhanced GABA release, future efforts should focus on the role of chronic ethanol in ethanol-enhanced GABA release.

If future pharmacotherapy options for alcohol use disorders are based upon a change in GABA release, the strategy will likely involve modulation of a GPCR, as do approximately 30% of all marketed prescription drugs (Hopkins and Groom, 2002). Naltrexone, which is one of the drugs approved for the treatment of alcohol dependence, acts at a GPCR. Naltrexone reduces alcohol intake and craving by blocking the rewarding effects of alcohol (Volpicelli et al., 1995), and there is evidence that this mechanism involves modulation of GABA release. Specifically, naltrexone reverses the ethanol-induced increase in dopamine signaling in the mesolimbic pathway, suggesting that opioids are a mediator in the ethanol–dopamine interaction (Benjamin et al., 1993). Importantly, opioids do not to affect VTA dopamine neurons directly but instead hyperpolarize GABAergic interneurons (i.e. disinhibition) that provide inhibitory signals to the VTA dopamine neurons (Johnson and North, 1992). Overall, these data suggest that increasing the amount of GABA released onto the VTA dopamine neurons can reduce the rewarding properties associated with dopamine signaling in the mesolimbic pathway.

In addition to naltrexone, there are other drugs targeting GPCRs that have the potential to be new treatment options. In alcohol-dependent individuals, prazosin, an antagonist at the $G\alpha_q$-linked α_1 adrenergic receptor, reduces the number of drinking days (Simpson et al., 2009). Likewise, LY686017, an antagonist at the $G\alpha_q$-linked neurokinin 1 receptor, reduces alcohol craving in alcohol-dependent individuals (George et al., 2008). Baclofen, a $GABA_B$ receptor agonist, promotes abstinence, prevents relapse, and reduces withdrawal in individuals with an alcohol use disorder (Addolorato et al., 2002, 2007), but these results have not always been reproducible (Garbutt et al., 2007). Baclofen can be taken by alcohol-dependent individuals who also have cirrhosis of the liver, which is a population without many treatment options due to extensive liver metabolism of most drugs (Addolorato et al., 2007).

Overall, these data demonstrate that pharmacological manipulation of GPCR signaling is a promising strategy for treating alcohol use disorders, and different GPCR agonists and antagonists should continue to be pursued as treatment options, especially the drugs that target the GPCRs that affect GABA release. While the intracellular messengers located downstream from the GPCRs could possibly be targeted in certain circumstances, the promiscuity of these second messengers will likely limit their use.

8. Summary

In addition to any postsynaptic actions, evidence is provided that ethanol increases GABA release in multiple brain regions. The ability of ethanol to increase GABA release can be influenced by GPCRs and the intracellular messengers located downstream from the GPCRs. In the CeA and the VTA, activation of a GPCR is required for ethanol to increase GABA release; therefore, future work should focus on determining if activation of other GPCRs is required for ethanol to increase GABA release in other brain regions. The way in which ethanol interacts with the GPCR to increase GABA release is uncertain, but it likely does not involve ethanol binding directly to the GPCR. Ethanol does not increase GABA release in every brain region, and this circumstance could potentially be explained by whether or not there is activation of a GPCR pathway at a GABAergic synapse or if the GABA released at a synapse is dependent on calcium release from internal stores. Overall, significant progress has been made in exploring the mechanism of ethanol-enhanced GABA release, and it has been clearly established that GPCRs play an important role in this ethanol mechanism and in the treatment of alcohol use disorders. To expand upon the potential relevance of this ethanol effect on GABA release to alcohol dependence, future work should focus on whether chronic ethanol exposure influences ethanol-enhanced GABA release and if GPCRs contribute to any of the observed differences.

Abbreviations

cAMP	3′-5′-cyclic adenosine monophosphate
5-HT$_{2C}$	5-hydroxytryptamine 2C
ATP	adenosine-5′-triphosphate
baf A$_1$	bafilomycin A$_1$
CB1	cannabinoid-1
CeA	central nucleus of the amygdala
CRF	corticotropin-releasing factor
DAG	diacylglycerol
eIPSC	evoked inhibitory postsynaptic current
GABA	gamma-aminobutyric acid
GPCRs	G protein-coupled receptors
IP$_3$	inositol 1,4,5-trisphosphate
mIPSC	miniature inhibitory postsynaptic current
PPR	paired pulse ratio
PCPA	parachlorophenylalanine
PLC	phospholipase C

PKA	protein kinase A
PKC	protein kinase C
RyR	ryanodine receptor
SERCA	*sarco*(endo)plasmic reticulum Ca^{2+} ATPase
sIPSC	spontaneous inhibitory postsynaptic current
VTA	ventral tegmental area

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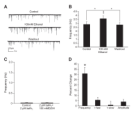

Fig. 1.

Ethanol increases vesicular release of GABA. A: A trace from a representative neuron illustrating the effect of ethanol (100mM) on mIPSC frequency. B: Ethanol significantly increased mIPSC frequency compared to control and washout [*p<.05; repeated measures ANOVA; F(2,42)=18.3]. The mIPSC frequency values for the control and washout groups were not significantly different. C: Incubating the slice with bafilomycin A_1 (baf A_1), which eliminates the pH and electrical gradients necessary for GABA transporters to fill the vesicles (Maycox et al., 1990), greatly reduced mIPSC frequency. In the presence of bafilomycin A_1, 100 mM ethanol did not significantly increase mIPSC frequency compared to control. D: Ethanol (100 mM) significantly increased mIPSC frequency (see B) in contrast to weak and non-significant effects on mIPSC fast decay time (τ-fast), slow decay time (τ-slow) and amplitude. Overall, these results support that the ethanol-induced increase in mIPSC frequency should be interpreted as an increase in spontaneous GABA release from the presynaptic terminal. This figure was reproduced from Kelm et al. (2010).

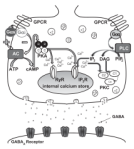

Fig. 2.

Ethanol, GABAergic neurotransmission, and GPCRs. Ethanol has been shown to act directly at the postsynaptic $GABA_A$ receptor, but ethanol can also increase the amount of GABA released from the presynaptic terminal. While the mechanism mediating this ethanol effect is not fully understood, evidence suggests that the GPCRs and their respective pathways are involved. It is unlikely that ethanol is binding directly to the GPCR and instead could be acting upstream of the receptor by increasing the amount of ligand reaching the receptor or changing the receptor properties. Ethanol could also interact directly with one of the intracellular messengers (see the text for a detailed description of the GPCR pathways).

Table 1

A summary of the slice electrophysiological techniques used in each brain region to measure the effect of ethanol on GABA release.

Neuronal type recorded	Type of GABA release measured	Ethanol effect	Reference
Basolateral amygdala (MDN)	mIPSCs	Increased mIPSC frequency	Zhu and Lovinger (2006)
Basolateral amygdala (MDN)	sIPSCs	Increased sIPSC frequency	Zhu and Lovinger (2006)
Basolateral amygdala	PPR	Decreased PPR	Silberman et al. (2008)
Brainstem motor	mIPSC	Increased mIPSC frequency	Sebe et al. (2003)
Central nucleus of the amygdala	mIPSC	Increased mIPSC frequency	Roberto et al. (2003), Kang-Park et al. (2007), Nie et al. (2009)
Central nucleus of the amygdala	PPR	Decreased PPR	Roberto et al. (2003), Nie et al. (2004)
Cerebellar Purkinje (MDN and slice)	mIPSCs	Increased mIPSC frequency	Ming et al. (2006), Kelm et al. (2007), Mameli et al. (2008), Hirono et al. (2009)
Cerebellar Purkinje (MDN and slice)	sIPSCs	Increased sIPSC frequency	Criswell et al. (2008), Mameli et al. (2008), Hirono et al. (2009)
Cerebellar Purkinje	PPR	Decreased PPR	Kelm et al. (2007), Criswell et al. (2008), Mameli et al. (2008)
Cerebellar granule	mIPSCs	Increased mIPSC frequency	Carta et al. (2004)
Cerebellar granule	sIPSCs	Increased sIPSC frequency	Carta et al. (2004)
Cerebrocortical (MDN)	sIPSCs	No effect	Criswell et al. (2008)
Hippocampal CA1 pyramidal	mIPSCs	Increased mIPSC frequency	Li et al. (2006)
Hippocampal CA1 pyramidal	sIPSCs	Increased sIPSC frequency	Ariwodola and Weiner (2004), Li et al. (2006)
Lateral septal (MDN)	sIPSCs	No effect	Criswell et al. (2008)
Lateral septal	PPR	No effect	Criswell et al. (2008)
Spinal cord motor	mIPSCs	Increased mIPSC frequency	Ziskind-Conhaim et al. (2003)
Spinal cord motor	sIPSCs	Increased sIPSC frequency	Cheng et al. (1999)
Substantia nigra (MDN)	mIPSCs	Increased mIPSC frequency	Criswell et al. (2008)
Thalamocortical relay	sIPSCs	No effect	Jia et al. (2008)
VTA	mIPSCs	Increased mIPSC frequency[1] and no effect[2]	1: Theile et al. (2008), 2: Xiao and Ye (2008)[2]
VTA	sIPSCs	Increased sIPSC frequency	Theile et al. (2008), Xiao and Ye (2008)[a]
VTA	PPR	Decreased PPR	Theile et al. (2008)

[a]Note: The increase in sIPSC frequency was only seen in the presence of a μ-opioid receptor agonist. MDN: mechanically dissociated neurons.

Table 2

A summary of the role of GPCRs in ethanol-enhanced GABA release.

GPCR	Neuronal type recorded	G Protein	Effect on ethanol-enhanced GABA release*	Effect on baseline GABA release	Reference
CB1 receptor	Basolateral amygdala	$G\alpha_i$	Agonist blocked ethanol effect; antagonist facilitated ethanol effect	Not reported	Talani and Lovinger (2008)
CB1 receptor	CeA	$G\alpha_i$	Agonist blocked ethanol effect	Agonist decreased GABA release	Roberto et al. (2008)
CB1 receptor	Cerebellar Purkinje	$G\alpha_i$	Agonist blocked the ethanol effect[1], while the antagonist had no effect[2]	Agonist decreased GABA release[1]	1: Kelm et al., 2008; 2: Kelm et al., 2007
CRF1 receptor	CeA	$G\alpha_s$	Antagonist blocked the ethanol effect	Not reported	Nie et al. (2004)
δ-Opioid receptor	CeA	$G\alpha_i$	Antagonist enhanced the ethanol effect	Antagonist had no effect on GABA release	Kang-Park et al. (2007)
$GABA_B$ receptor	Basolateral amygdala	$G\alpha_i$	Antagonist enhanced the ethanol effect[1,2], and agonist blocked the ethanol effect[2]	Antagonist had no effect on spontaneous GABA release[1,2]; antagonist decreased evoked GABA release at local synapses[2]	1: Zhu and Lovinger, 2006; 2: Silberman et al., 2009
$GABA_B$ receptor	Cerebellar Purkinje	$G\alpha_i$	Agonist blocked the ethanol effect, while the antagonist had no effect	Agonist decreased GABA release; antagonist increased GABA release	Kelm et al. (2007), Kelm et al. (2008)
$GABA_B$ receptor	Hippocampal CA1 pyramidal	$G\alpha_i$	Antagonist enhanced the ethanol effect	Antagonist had no effect on GABA release	Ariwodola and Weiner (2004)
$GABA_B$ receptor	VTA	$G\alpha_i$	Agonist and antagonist did not affect the ethanol effect	Agonist decreased GABA release; antagonist increased GABA release	Theile et al. (2008)
μ-Opioid receptor	CeA	$G\alpha_i$	Antagonist and null mice did not affect the ethanol effect	Antagonist increased GABA release; GABA release greater in null mice	Kang-Park et al. (2009)
μ-Opioid receptor	VTA	$G\alpha_i$	Ethanol only increased GABA release in the presence of agonist	Agonist decreased GABA release	Xiao and Ye (2008)
Nociceptin/orphani FQ peptide receptor	CeA	$G\alpha_i$	Agonist blocked ethanol effect	Agonist decreased GABA release	Roberto and Siggins (2006)
Serotonin $5\text{-}HT_{2C}$ receptor	VTA	$G\alpha_q$	Antagonist blocked ethanol effect	Antagonist had no effect on GABA release	Theile et al. (2009)

*For each experiment, the antagonist or agonist was applied before the application of ethanol and remained present through the ethanol application to determine the effect of the agonist/antagonist on ethanol-enhanced GABA release.