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Differential effects of $GABA_B$ autoreceptor activation on ethanol potentiation of local and lateral paracapsular GABAergic synapses in the rat basolateral amygdala

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ABSTRACT

Many studies have demonstrated that GABAergic inhibition within the basolateral amygdala (BLA) plays an integral role in the regulation of anxiety, an important behavioral component in the etiology of alcoholism. Although ethanol has recently been shown to enhance BLA GABAergic inhibition via two distinct populations of inhibitory cells, local and lateral paracapsular (lpcs) interneurons, little is known about the mechanisms underlying ethanol potentiation of these two inhibitory pathways. Ethanol is known to enhance GABAergic inhibition in many brain regions via a complex array of pre- and post-synaptic mechanisms. In addition, ethanol's presynaptic effects are often subject to $GABA_B$ autoreceptor ($GABA_B$-R) modulation. Therefore, in this study, we characterized $GABA_B$-R function and modulation of ethanol actions at local and lpcs GABAergic synapses. At local synapses, we found significant paired-pulse depression (PPD, 250 ms inter-pulse interval) which was abated by SCH-50911 ($GABA_B$-R antagonist). No significant PPD was detected at lpcs synapses, but SCH-50911 significantly potentiated lpcs-evoked IPSCs. Baclofen ($GABA_B$-R agonist) had similar depressant effects on local- and lpcs-evoked IPSCs, however baclofen pretreatment only reduced ethanol potentiation at local synapses. Ethanol also significantly enhanced the frequency of spontaneous and miniature IPSCs, and these effects were also sensitive to $GABA_B$-R modulators. Collectively, these data suggest that stimulus-independent inhibitory responses recorded from BLA principal neurons primarily reflect the activity of local GABAergic interneurons and provide additional evidence that ethanol potentiates local BLA inhibitory synapses primarily via a presynaptic enhancement of GABA release that is tightly regulated by $GABA_B$-Rs. In contrast, ethanol potentiation of lpcs GABAergic synapses is not sensitive to $GABA_B$-R activation and does not appear to involve increased presynaptic GABA release.

1. Introduction

Converging lines of clinical, behavioral, and pharmacological evidence suggest that the relationship between ethanol (EtOH) drinking and anxiety disorders may be an important component in the development and maintenance of alcoholism (Kushner et al., 2000). Indeed, alcohol use disorders are often found to be more prevalent in patients with many types of stress and anxiety disorders, such as generalized anxiety disorder (Grant et al., 2005), social anxiety (Carrigan and Randall, 2003), panic disorder (Cosci et al., 2007), or posttraumatic stress disorder (Stewart, 1996). In addition, stress and anxiety are also known to be important mediators in relapse to alcoholism (Breese et al., 2005). In the preclinical setting, it has been shown that episodes of stress can substitute for initial cycles of EtOH withdrawal in a behavioral model that increases EtOH-withdrawal anxiety in rats (Breese et al., 2004). Similarly, exposure to stress has been shown to increase anxiety and alcohol craving in recently abstinent human alcoholics (Fox et al., 2007), a response that was greater in those dependent on alcohol than in social drinkers (Sinha et al., in press). While the exact etiology of this connection between anxiety and alcoholism is not clearly defined, determining the neuropharmacological effects of EtOH in brain regions associated with anxiety-like behaviors may lead to a better understanding of the mechanisms underlying the development of alcoholism.

One brain region that is thought to play a critical role in the regulation of anxiety-like behaviors is the basolateral complex of the amygdala (BLA) (LeDoux, 1996). Located in the medial temporal

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lobe, the entire amygdaloid complex is comprised of many structurally diverse nuclei. Together, the lateral, basal, and accessory basal nuclei form the main portions of the BLA. The BLA receives sensory information from cortical and subcortical areas, integrates these inputs, and then projects to the central nuclei of the amygdala and other brain regions, ultimately eliciting the appropriate behavioral response to external stimuli. As such, the BLA is thought to play an important role in controlling the output of processed information from the entire amygdaloid complex (Sah et al., 2003). Glutamatergic pyramidal cells comprise ~85–95% of the neurons within the BLA, while the remaining cells are thought to be GABAergic interneurons (Washburn and Moises, 1992). Typically, increases in BLA excitation are associated with elevated levels of a broad spectrum of anxiety-like behaviors while decreases in BLA activity are usually associated with anxiolysis (Davis et al., 1994). Although GABAergic interneurons only comprise a small portion of the total neurons in this brain region, these cells are thought to play a critical role in regulating BLA excitability and the appropriate output of the region to other structures and thus are likely to play an integral role in regulating anxiety-like behaviors.

The importance of BLA GABAergic transmission in regulating anxiety has been established in both the clinical and the preclinical settings. Preclinically, numerous studies have shown that direct infusion of $GABA_A$ receptor modulators into the BLA can alter some measures of anxiety-like behaviors (Bueno et al., 2005; Sanders and Shekhar, 1995). In the clinical setting, fMRI studies have shown BLA activity to be correlated with trait anxiety levels (Etkin et al., 2004). Furthermore, many drugs that can enhance GABAergic synaptic transmission in the BLA are often the first line of pharmacotherapy used to treat anxiety disorders (Allison and Pratt, 2003), thus implicating increased BLA inhibition in the treatment of anxiety. Interestingly, many of the acute pharmacological properties of EtOH may be attributable to an enhancement of GABAergic synaptic transmission (Criswell and Breese, 2005; Weiner and Valenzuela, 2006), leading to the hypothesis that EtOH enhancement of BLA GABAergic transmission may contribute to its well-known anxiolytic properties. Therefore, determining the mechanisms through which EtOH alters GABAergic synaptic transmission in the BLA may provide insight into the pathophysiology of alcoholism and provide new avenues for the development of pharmacotherapies to help alleviate this disease.

Previous research from our lab and others has shown that pyramidal cells in the BLA receive inhibitory input from at least two distinct populations of GABAergic interneurons, distinguishable by their pharmacological and morphological properties (Fuxe et al., 2003; Marowsky et al., 2005; Silberman et al., 2008). The first group of cells is comprised mainly of local circuit interneurons, which are sparsely dispersed throughout the BLA, stain positive for typical interneuronal markers such as parvalbumin, and are thought to mediate local feed-back inhibition (Marowsky et al., 2005; McDonald and Mascagni, 2001). Recently, a second group of interneurons have been characterized in the BLA: the lateral paracapsular cells (lpcs). These cells are located in dense clusters along the BLA-external capsule border, do not stain for parvalbumin or other classical interneuron markers such as CCK or calbindin, and are thought to mediate feed-forward inhibition from cortical inputs (Marowsky et al., 2005). Importantly, while EtOH potentiates inhibitory postsynaptic currents (IPSCs) evoked from both local and lpcs interneurons to a similar extent, the mechanisms underlying EtOH enhancement of these two groups of interneurons appear to be distinct.

We have recently shown that EtOH potentiation of locally-evoked IPSCs is associated with a decrease in paired-pulse ratio and is enhanced by pretreatment with a $GABA_B$ receptor antagonist, SCH-50911 (Silberman et al., 2008). This confirms earlier findings that SCH-50911 can enhance EtOH's ability to increase spontaneous IPSC frequency in isolated synaptic boutons from BLA pyramidal cells (Zhu and Lovinger, 2006). Collectively, these findings suggest that EtOH enhancement of local interneuronal inhibition in the BLA may be primarily due to a presynaptic mechanism that can be regulated by $GABA_B$ autoreceptors. In contrast, EtOH potentiation of lpcs IPSCs was not associated with a change in paired-pulse ratio and was not altered by pretreatment with SCH-50911. Interestingly SCH-50911 application alone significantly potentiated lpcs IPSCs while having no effect on local IPSCs. These findings suggest that EtOH facilitation of lpcs IPSCS may not be mediated by an increase in GABA release probability and, importantly, that there may be significant differences in presynaptic $GABA_B$ receptor sensitivity or activity between local and lpcs synapses. Therefore, in this study, we sought to further characterize presynaptic $GABA_B$ autoreceptor regulation of GABA release in these two distinct inhibitory pathways in the BLA. Our second goal was to utilize the $GABA_B$ receptor system to further characterize the distinct mechanisms by which EtOH enhances GABAergic inhibition in the BLA. Our findings reveal significant differences in synaptic, but not exogenous agonist, activation of $GABA_B$ receptors at local and lpcs BLA GABAergic synapses. Our findings also provide further evidence that EtOH facilitation of local IPSCs is mediated by a presynaptic facilitation of GABA release that is under the tight regulation of $GABA_B$ autoreceptors. In contrast, our data suggest that EtOH enhancement of lpcs synaptic transmission does not result from an increase in GABA release and is not modulated by synaptic or exogenous agonist-mediated activation of $GABA_B$ receptors.

2. Methods

2.1. Slice preparation

Transverse amygdala slices (400 μm) were prepared from 4- to 6-week-old male Sprague Dawley rats. Slices were maintained at ambient temperature for at least 2 h in oxygenated artificial cerebrospinal fluid (aCSF) containing (in mM): 124 NaCl, 3.3 KCl, 2.4 $MgCl_2$, 2.5 $CaCl_2$, 1.2 KH_2PO_4, 10 D-glucose, and 25 $NaHCO_3$, saturated with 95% O_2 and 5% CO_2.

2.2. Electrophysiological recordings

Slices were transferred to a recording chamber and superfused with aerated aCSF at 2 ml/min using a calibrated flowmeter (Gilmont Instruments, Racine, WI). Experiments were performed at ambient temperature as our previous studies have found that this promotes the stability of patch-clamp recordings in brain slices and does not influence EtOH enhancement of $GABA_A$ IPSCs (Ariwodola and Weiner, 2004). Recording electrodes were prepared from filamented borosilicate glass capillary tubes (outer diameter: 1.5 mm, inner diameter: 0.86 mm) using a horizontal micropipette puller (P-97; Sutter Instruments, Novato, CA). Whole-cell patch-clamp recordings of evoked IPSCs (eIPSCs) were made using a filling solution containing 130 mM K-gluconate, 10 mM KCl, 1 mM EGTA, 100 μM $CaCl_2$, 2 mM Mg-ATP, 200 μM Tris–guanosine 5′-triphosphate, and 10 mM HEPES, pH adjusted with KOH, 275–280 mOsm. Recordings of spontaneous IPSCs (sIPSCs) and TTX-resistant miniature IPSCS (mIPSCs) were made using a similar filling solution exchanging equimolar CsCl for K-gluconate and KCl. In all experiments, 5 mM N-(2,6-dimethylphenylcarbamoylmethyl)-triethylammonium chloride (QX-314) was included in the recording solution to block voltage-gated sodium currents and $GABA_B$ IPSCs in the BLA neurons being recorded (Horn et al., 1980; Nathan et al., 1990). Whole-cell patch-clamp recordings were made from BLA pyramidal neurons voltage-clamped at −30 to −40 mV for eIPSCs and at −60 to −70 mV for s/mIPSCs (not corrected for junction potential). Only cells with a stable access resistance of 5–20 MΩ were used in these experiments. Whole-cell currents were acquired using an Axoclamp 2B or Axopatch 200B amplifier, digitized (Digidata1200 or Digidata 1321A; Axon Instruments, Union City, CA), and analyzed on- and off-line using an IBM-compatible personal computer and pClamp 9.0 software (Axon Instruments).

2.3. Pharmacological isolation of synaptic currents

In many experiments, $GABA_A$ IPSCs were evoked every 20 s by electrical stimulation (0.2 ms duration) using a concentric bipolar stimulating electrode (FHC, Bowdoinham, ME) placed near (50–100 μm) the recording electrode to target local interneurons (“local” stimulation) or along the external capsule to target lpcs interneurons (“distal” stimulation). In some experiments designed to assess the