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Neurobiological mechanisms contributing to alcohol–stress–anxiety interactions

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Abstract

This article summarizes the proceedings of a symposium that was presented at a conference entitled "Alcoholism and Stress: A Framework for Future Treatment Strategies." The conference was held in Volterra, Italy on May 6–9, 2008 and this symposium was chaired by Jeff L. Weiner. The overall goal of this session was to review recent findings that may shed new light on the neurobiological mechanisms that underlie the complex relationships between stress, anxiety, and alcoholism. Dr. Danny Winder described a novel interaction between D1 receptor activation and the corticotrophin-releasing factor (CRF) system that leads to an increase in glutamatergic synaptic transmission in the bed nucleus of the stria terminalis. Dr. Marisa Roberto presented recent data describing how protein kinase C epsilon, ethanol, and CRF interact to alter GABAergic inhibition in the central nucleus of the amygdala. Dr. Jeff Weiner presented recent advances in our understanding of inhibitory circuitry within the basolateral amygdala (BLA) and how acute ethanol exposure enhances GABAergic inhibition in these pathways. Finally, Dr. Brian McCool discussed recent findings on complementary glutamatergic and GABAergic adaptations to chronic ethanol exposure and withdrawal in the BLA. Collectively, these investigators have identified novel mechanisms through which neurotransmitter and neuropeptide systems interact to modulate synaptic activity in stress and anxiety circuits. Their studies have also begun to describe how acute and chronic ethanol exposure influence excitatory and inhibitory synaptic communication in these pathways. These findings point toward a number of novel neurobiological targets that may prove useful for the development of more effective treatment strategies for alcohol use disorders.

Keywords: Acute; Basolateral amygdala; Bed nucleus of the stria terminalis; Central nucleus of the amygdala; Chronic ethanol; CRF; Dopamine; GABA; Glutamate; Withdrawal

Introduction

There is a large and growing body of clinical and preclinical evidence suggesting an important, albeit complex, relationship between stress, anxiety, and alcohol use disorders (AUDs) (Kushner et al., 2000a; Piazza and Le Moal, 1998; Roberts et al., 2000; Weiss et al., 2001). For example, clinical studies have documented a significant degree of comorbidity between anxiety disorders and AUDs (Kessler et al., 1997; Kushner et al., 1999; Regier et al., 1990). Furthermore, ethanol dependence is often viewed as a chronic relapsing disease (Heilig and Egli, 2006) and there is evidence that stress and anxiety may promote relapse and negatively influence treatment prognosis (Fox

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et al., 2007; Kushner et al., 2005; Miller and Harris, 2000; Sinha and Li, 2007; Willinger et al., 2002).

Although these and many other studies consistently report a strong association between anxiety and AUDs (see Bradizza et al., 2006; Cosci et al., 2007; Kushner et al., 2000a), the etiological nature of this relationship is not well understood. However, recent preclinical findings are beginning to shed light on this clinically important topic. Human and animal studies have shown that acute exposure to low-to-moderate doses of ethanol are anxiolytic (see Koob, 2004; Kushner et al., 2000a for reviews) and repeated exposure and withdrawal are associated with neuroadaptive changes that may lead to persistent increases in a range of anxiety measures (Kliethermes, 2005; Roberts et al., 2000; Santucci et al., 2008; Valdez et al., 2002). Several studies have also shown that, during withdrawal, ethanol-exposed animals display significant increases in voluntary ethanol consumption (Becker and Lopez, 2004; Lopez and Becker, 2005; Roberts et al., 1996). Moreover, increased intake in ethanol-dependent animals can be effectively reduced by treatments that can attenuate withdrawal-associated anxiety (e.g., CRF1-R [receptor] antagonists) (Chu et al., 2007; Roberts et al., 1995; Valdez et al., 2002). These and other recent findings have led to the recognition that ethanol use and abuse likely involve both the positive and negative reinforcing effects of this drug (Koob and Le Moal, 2005). Early on, the positive or euphoric effects of ethanol (associated with the classical activation of the mesolimbic reward circuit) may dominate. However, following prolonged ethanol exposure and/or in some individuals with pre-existing anxiety disorders (Cosci et al., 2007; Kushner et al., 2000b), the negative reinforcing effects of ethanol, including anxiolysis, may become increasingly important and play a major role in both the development of abusive drinking behavior and in relapse (Koob and Le Moal, 2008; Le Moal and Koob, 2007; Lopez and Becker, 2005).

Interestingly, although much is known about the basic neurophysiological mechanisms underlying ethanol's positive reinforcing effects, the neural substrates responsible for the negative reinforcing effects of this drug (including relief from anxiety) are much less understood. To that end, this symposium sought to highlight recent advances in our understanding of how synaptic communication in brain regions that regulate stress- and anxiety-related behaviors (e.g., amygdala, bed nucleus of the stria terminalis) can be modulated by endogenous factors such as dopamine and corticotrophin-releasing factor (CRF) as well as acute and chronic ethanol.

Ethanol and CRF: which is driving GABA release in the amygdala?

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CRF is an anxiogenic neuropeptide and an important component of the stress circuits that modulate anxiety associated with drug dependence. The anxiogenic effects of CRF are mediated by type 1 CRF receptors (CRF-R1s), which are abundantly expressed in the cortex, cerebellum, hippocampus, amygdala, olfactory bulb, and pituitary (Chalmers et al., 1996; Palchaudhuri et al., 1998; Potter et al., 1994). CRF-R1 activation also plays an important role in regulating voluntary ethanol intake. The central nucleus of the amygdala (CeA) is a pivotal site of action for both the acute positive reinforcement of ethanol addiction and for the negative reinforcement associated with ethanol abstinence (Koob and Le Moal, 2001). CRF release in the CeA is increased in alcohol-dependent animals (Merlo Pich et al., 1995; Olive et al., 2002) and appears to contribute to alcohol withdrawal-related anxiety, which can be reduced by CRF-R1 receptor antagonists injected into the CeA (Rassnick et al., 1993). CRF also contributes to increased alcohol consumption in dependent animals because their increased ethanol self-administration is reduced by CRF-R1 antagonists (Funk et al., 2007; Overstreet et al., 2004) or the deletion of the CRF-R1 (Chu et al., 2007).

GABAergic transmission in the CeA has been implicated in regulating ethanol intake (Hyytia and Koob, 1995; Roberto et al., 2004). Most of the neurons in the rodent CeA are GABAergic inhibitory neurons with inhibitory recurrent or feed-forward connections, as well as inhibitory projections to brainstem nuclei (Cassell et al., 1999; Sun and Cassell, 1993). CRF is abundant in the CeA, where it is coexpressed with GAB0A (Day et al., 1999). We have previously shown that CRF and ethanol enhance GABA release from mouse CeA neurons in a CRF-R1-dependent manner (Nie et al., 2004). However, little is known about the cellular mechanisms through which GABA transmission in the CeA modulates the behavioral and motivational effects of CRF and ethanol.

Recent in vitro evidence indicates that protein kinase C (PKC) signaling is stimulated by CRF-R1 activation (Kageyama et al., 2007; Kim et al., 2007). PKC is a family of serine–threonine kinases that respond to lipid second messengers and have been implicated in neurobehavioral disorders, including anxiety and drug abuse (Olive and Messing, 2004). Among the PKC isozymes, we hypothesized that protein kinase C epsilon (PKCϵ) mediates downstream effects of CRF-R1 activation in the CeA because PKCϵ is expressed throughout the amygdala (Choi et al., 2002), and PKCϵ$^{-/-}$ mice show reduced anxiety-like behavior (Hodge et al., 2002) and reduced alcohol consumption (Hodge et al., 1999; Olive et al., 2000). To test this hypothesis, we studied the role of PKCϵ signaling in basal CeA GABAergic transmission and in ethanol- and CRF-induced GABA release in an in vitro slice preparation using both genetic and pharmacological approaches (Bajo et al., 2008). Here, we examined signaling pathways downstream of the CRF-R1 in the CeA that mediate GABAergic signaling and anxiety. We characterized the effects of acute ethanol and CRF on CeA GABAergic synapses in mice with a null mutation for PKCϵ (PKCϵ$^{-/-}$) and wild-type (PKCϵ$^{+/+}$) littermates.