Cold Spring Harbor Perspectives in Medicine
www.perspectivesinmedicine.org

The Central Amygdala and Alcohol: Role of γ-Aminobutyric Acid, Glutamate, and Neuropeptides

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Alcohol dependence is a chronically relapsing disorder characterized by compulsive drug seeking and drug taking, loss of control in limiting intake, and the emergence of a withdrawal syndrome in the absence of the drug. Accumulating evidence suggests an important role for synaptic transmission in the central amygdala (CeA) in mediating alcohol-related behaviors and neuroadaptative mechanisms associated with alcohol dependence. Acute alcohol facilitates γ-aminobutyric acid-ergic (GABAergic) transmission in CeA via both pre- and postsynaptic mechanisms, and chronic alcohol increases baseline GABAergic transmission. Acute alcohol inhibits glutamatergic transmission via effects at *N*-methyl-D-aspartate (NMDA) and AMPA receptors in CeA, whereas chronic alcohol up-regulates *N*-methyl-D-aspartate receptor (NMDAR)-mediated transmission. Pro- (e.g., corticotropin-releasing factor [CRF]) and anti-stress (e.g., NPY, nociceptin) neuropeptides affect alcohol- and anxiety-related behaviors, and also alter the alcohol-induced effects on CeA neurotransmission. Alcohol dependence produces plasticity in these neuropeptide systems, reflecting a recruitment of those systems during the transition to alcohol dependence.

Alcoholism (i.e., dependence on alcohol) is a complex and dynamic disease process. Alcohol dependence is a chronically relapsing disorder characterized by (1) compulsive drug seeking and drug taking, (2) loss of control in limiting intake (in terms of amount of drug per bout and number of drug-taking bouts), and (3) the emergence of a withdrawal syndrome in the absence of the drug that includes, but is not limited to, dysphoria, sleep disturbances, disruption of autonomic processes, and increases in anxiety and irritability (ICD-10 and DSM-IV). Many years of research have shaped the current view that excessive alcohol consumption is largely mediated by an organism's past experience with alcohol (e.g., intake pattern and frequency), and is driven by the emotional disturbances, rather than the physical

Editors: R. Christopher Pierce and Paul J. Kenny
Additional Perspectives on Addiction available at www.perspectivesinmedicine.org

 doi: 10.1101/cshperspect.a012195
Cite this article as *Cold Spring Harb Perspect Med* 2012;2:a012195

disturbances, associated with alcohol withdrawal and abstinence (Koob 2003; Heilig et al. 2010). Although the central amygdala (CeA) is known to play a role in such negative affective alcohol responses, the neuronal circuitry underlying these behavioral stages is still under considerable scrutiny. This article will focus on neurotransmission in the central amygdala and its role in driving the negative affect characteristic of the withdrawal phase of alcohol addiction. Throughout this article, *acute* alcohol exposure refers to in vitro application of alcohol onto the slice preparation, whereas *chronic* alcohol exposure refers to long-duration (at least several weeks) in vivo alcohol exposure.

CENTRAL AMYGDALA IS A HUB FOR NEGATIVE EMOTIONAL CIRCUITRY

Chronic consumption of large quantities of drugs, including alcohol, promotes a transition from casual drug use to drug dependence that is defined in part by down-regulation of dopamine signaling in the mesocorticolimbic reward system, hyperactivity of glutamate signaling, and dysregulation of brain stress systems (Koob and Volkow 2010). Chronic alcohol effects on brain stress systems can refer to either alcohol-induced changes in neuroendocrine function (i.e., hypothalamic-pituitary-adrenal [HPA] axis; Kiefer and Wiedemann 2004; Clarke et al. 2008) or the recruitment of extrahypothalamic brain stress systems such as the amygdala (Koob 2009). The present article details the effects of acute and chronic alcohol on synaptic transmission and plasticity in the CeA and neighboring regions, and the role of these regions in mediating alcohol-related behaviors. We also will review the literature on peptidergic modulation of inhibitory and excitatory transmission in the central (and extended) amygdala, because these peptides share a common cellular target and interact with each other and alcohol.

Many of the long-term emotional disturbances associated with alcohol abuse and dependence are attributed to neurotransmission within a conceptual macrostructure in the basal forebrain called the "extended amygdala" (Koob 2008). In the context of drug addiction, the major constituents of the extended amygdala are the CeA, the lateral portion of the bed nucleus of stria terminalis (BNST), and nucleus accumbens (NAc) shell (Heimer and Alheid 1991). These regions show similar morphology, a high degree of interconnectivity, and overlapping afferents from limbic cortices, hippocampus, and basolateral amygdala (BLA). The outputs of the extended amygdala project largely to effector regions, including lateral hypothalamus and various brain stem regions, that produce behaviors related to fear and anxiety (Davis et al. 2010).

The role of the extended amygdala in fear and anxiety has been previously described in detail (Ciocchi et al. 2010; Davis et al. 2010). The CeA and BNST are integral in mediating fear and anxiety responses. The BLA receives significant sensory input from thalamus and cortex, sends prominent glutamatergic projections to CeA and BNST, and is integral in both conditioning (Phelps and LeDoux 2005) and extinction (Quirk and Mueller 2008) processes. The CeA is composed mostly of γ-aminobutyric acid-ergic (GABAergic) projection neurons and interneurons (Sun and Cassell 1993; Veinante and Freund-Mercier 1998), and the BNST is a major target of CeA projection neurons (Krettek and Price 1978; Weller and Smith 1982; Sun and Cassell 1993; Veinante and Freund-Mercier 1998). Of major relevance for this article, connections between CeA and BNST often contain neuropeptide cotransmitters. For example, the CeA is a major source of corticotropin-releasing factor (CRF) in the BNST (Sakanaka et al. 1986). Therefore, the CeA is uniquely situated to convert sensory information into behavioral and physiological responses, and this is particularly true for stress- and alcohol-related stimuli.

Neuropeptides in the CeA are important for producing the negative affective state observed during withdrawal from drugs, including alcohol (Koob 2008). Here we will describe alcohol-induced neuroadaptations in select peptidergic systems (CRF, nociception, and NPY), largely in the context of the CeA. It is becoming increasingly evident that these peptides interact in complex ways in the CeA to modulate

GABAergic inhibitory and glutamatergic excitatory transmission, and that dysregulation of these peptide systems by alcohol alters the way in which they modulate such neurotransmission. Conceptually, these neuropeptides have been divided into prostress peptides and antistress peptides that respectively, promote and rescue negative affective disturbances during drug abstinence following heavy drug use. Prostress peptides include CRF, dynorphin, orexin, and vasopressin, whereas antistress peptides include neuropeptide Y (NPY), enkephalin, and nociceptin; however, owing to space limitations we will discuss only a select few of these peptides.

AMYGDALAR GABAergic SYSTEM AND ALCOHOL

GABA, the major inhibitory transmitter in the brain, acts on two classes of GABA receptors: $GABA_A$ (which includes $GABA_A$-rho subclass—formerly $GABA_C$) and $GABA_B$. $GABA_A$ receptors are ligand-gated ion channels, whereas $GABA_B$ receptors are G protein coupled. There is considerable evidence that GABAergic transmission mediates some aspects of alcohol-drinking behavior, but there is ambiguity in the literature with respect to the directions of these effects. Early studies showed that systemic administration of $GABA_AR$ agonists increased voluntary alcohol drinking, whereas $GABA_AR$ antagonists and benzodiazepine inverse agonists decreased alcohol drinking (Boyle et al. 1993; Rassnick et al. 1993). Infusion of both $GABA_AR$ agonists and antagonists into the nucleus accumbens (NAcc) suppressed alcohol drinking by nondependent rats (Hodge et al. 1995). Systemic administration of a $GABA_BR$ agonist suppressed alcohol drinking by all rats, but alcohol-dependent rats were more sensitive to this effect, suggesting an up-regulation of $GABA_BR$ function (Walker and Koob 2007). Other studies highlight a role for GABA circuitry in alcohol-drinking behavior, particularly in regions implicated in the negative reinforcing properties of the drug (i.e., extended amygdala). Hyytia and Koob (Hyytia and Koob 1995) found that injection of $GABA_AR$ antagonists in the three major regions of the extended amygdala suppressed alcohol drinking by nondependent rats, but this effect was most potent and selective for alcohol when infused into the CeA. Another study showed that antagonism of $GABA_ARs$ in the BNST reversed decreases in alcohol drinking elicited by a D_2 receptor antagonist infused into the ventral tegmental area (VTA) of alcohol-preferring (P) rats (Eiler and June 2007). Interestingly, infusion of a $GABA_AR$ agonist directly into the amygdala suppresses drinking by alcohol-dependent rats without affecting intake by nondependent controls (Roberts et al. 1996). Although there are considerable methodological differences between these studies, they suggest that (1) chronic alcohol produces neuroadaptations in GABAergic neurotransmission and changes sensitivity to GABAergic compounds, (2) GABAergic neurotransmission regulates alcohol drinking, and (3) in the case of excessive alcohol consumption by alcohol-dependent rats, the CeA is a strong candidate region for localization of these effects.

ACUTE ALCOHOL AUGMENTS GABAergic TRANSMISSION IN CeA

The acute effects of alcohol on $GABA_AR$ function has been extensively studied, mostly in in vitro brain slice preparations, an approach that allows multiple methods for detecting changes in presynaptic transmitter release (for a review, see Criswell and Breese 2005; Siggins et al. 2005; Weiner and Valenzuela 2006; Lovinger and Roberto 2011). Alcohol (1–100 mM) selectively enhances the function of $GABA_ARs$ containing certain subunit compositions, but such findings have been inconsistent across laboratories testing heterologous systems (reviewed in Aguayo et al. 2002; Lovinger and Homanics 2007). Alcohol increases GABAergic synaptic transmission in the CeA (Roberto et al. 2003) and BLA (Zhu and Lovinger 2006) via increased presynaptic GABA release. Specifically, alcohol augments evoked inhibitory postsynaptic currents (IPSCs), decreases paired-pulse facilitation (PPF) of evoked IPSCs, and increases the frequency of miniature inhibitory postsynaptic currents (mIPSCs) (i.e., in Tetrodotoxin to

eliminate action potential firing) in most CeA neurons, suggesting that alcohol increases vesicular GABA release.

Although the molecular mechanism(s) for alcohol effects have yet to be identified, we showed that activation of adenylyl cylase (AC) and/or protein kinase C (PKC) mediate GABAergic transmission in CeA synapses (Bajo et al. 2008; Cruz et al. 2011). In CeA slices of mice lacking PKCε or pretreated with a PKCε antagonist, the ability of acute alcohol to augment IPSCs is impaired (Bajo et al. 2008), suggesting that PKCε facilitates alcohol-elicited vesicular GABA release. Notably, basal GABA release is greater in CeA of PKCε knockout than wild-type (WT) mice, suggesting that under normal conditions in WT neurons, PKCε limits spontaneous GABA release. Therefore, PKCε serves at least two roles in the CeA: (i) limiting baseline GABA release, and (ii) facilitating alcohol-stimulated release of GABA.

The ability of alcohol to facilitate GABA neurotransmission may be limited by GABA feedback onto presynaptic $GABA_B$Rs (Wan et al. 1996; Ariwodola and Weiner 2004). For example, acute alcohol facilitates GABAergic transmission in hippocampus (Wu and Saggau 1994; Wan et al. 1996; Ariwodola and Weiner 2004) and nucleus accumbens (Nie et al. 2000) only if $GABA_B$ receptors are blocked. However, in the CeA, $GABA_B$ receptor blockade is not required for the enhancement of IPSPs by acute alcohol nor does it potentiate this effect (Roberto et al. 2003). Thus, the involvement of $GABA_B$ receptors in alcohol-induced GABA release may depend on the presence of presynaptic $GABA_B$ receptors in certain brain regions (Ariwodola and Weiner 2004; Breese et al. 2005).

CHRONIC ALCOHOL EFFECTS ON GABAergic TRANSMISSION IN CeA

Our in vitro electrophysiological results show that chronic alcohol exposure augments GABA release in the CeA (Roberto et al. 2004a). Compared to alcohol-naïve controls, alcohol-dependent rats show larger baseline evoked $GABA_A$-mediated IPSP/C amplitudes, smaller baseline PPF of evoked IPSCs, and higher baseline frequency of mIPSCs in CeA neurons. Interestingly, acute alcohol augments IPSCs, decreases PPF of IPSCs, and increases mIPSC frequency similarly in alcohol-dependent and alcohol-naïve rats, suggesting a lack of tolerance for these acute effects of alcohol (Roberto et al. 2004a). In vivo microdialysis studies indicate a fourfold increase of baseline dialysate GABA concentrations in the CeA of alcohol-dependent rats relative to naïve controls, as well as lack of tolerance for acute alcohol-induced increases in dialysate GABA levels (Roberto et al. 2004a), strongly suggesting that both acute and chronic alcohol alter presynaptic elements of GABAergic synapses in the CeA. Future studies need to determine the molecular mechanisms responsible for chronic alcohol-induced adaptations in CeA neurons and their behavioral implications in alcohol-dependent and/or alcohol-withdrawn organisms. These ongoing studies may elucidate the mechanism(s) underlying reductions in alcohol withdrawal hyperexcitability produced by GABAmimetic drugs (Ticku and Burch 1980; McCown et al. 1985; Breese et al. 2006; Roberto et al. 2008), and could impact treatment of pathological alcohol-drinking behaviors.

Chronic alcohol exposure produces tolerance to many behavioral effects of the drug, including the anxiolytic, sedative, ataxic, and positive reinforcing effects (Kumar et al. 2004, 2009). Chronic alcohol also produces physical and motivational dependence, and alcohol withdrawal is associated with increased neuronal excitability in several brain regions (but not CeA) (Kliethermes 2005; Weiner and Valenzuela 2006). Chronic alcohol effects may reflect, in part, compensatory adaptations to the facilitatory effects of alcohol on GABAergic synapses (Siggins et al. 2005; Weiner and Valenzuela 2006). We showed that evoked IPSCs in CeA slices from alcohol-dependent rats are significantly larger than those from naive controls (Roberto et al. 2004a). Some CeA neurons from alcohol-dependent rats also show increased mIPSC amplitudes relative to naïve rats, suggesting a postsynaptic effect of chronic alcohol (Roberto et al. 2004a). Substantial evidence suggests that alcohol-induced behavioral and neural adaptations are attributable

to changes in $GABA_AR$ subunit composition rather than changes in the number of $GABA_ARs$ (Morrow et al. 1992; Eckardt et al. 1998; Grobin et al. 1998; Papadeas et al. 2001; Kumar et al. 2004, 2009).

In alcohol-naïve rats, a $GABA_BR$ antagonist increased the amplitude of evoked IPSCs and decreased PPF of IPSCs in CeA, suggesting tonic activation of presynaptic $GABA_BRs$ (Roberto et al. 2008). Conversely, a $GABA_B$ agonist markedly depressed evoked IPSC amplitudes and increased PPF of IPSCs in the CeA of alcohol-naïve rats, indicating decreased presynaptic GABA release. These effects of $GABA_BR$ agonists and antagonists were absent or greatly attenuated in the CeA of alcohol-dependent rats, suggesting chronic alcohol-induced down-regulation of the $GABA_BR$ system that may explain the increased GABAergic tone observed in the CeA of dependent rats (Roberto et al. 2008). These alcohol dependence-induced neuroadaptations of the $GABA_BR$ system also may account for chronic alcohol-induced changes in gabapentin effects on inhibitory transmission in CeA. Gabapentin, a structural analog of GABA (Sills 2006), increases the amplitudes of evoked IPSCs in CeA neurons from nondependent rats (an effect blocked by a $GABA_BR$ antagonist), but decreases IPSC amplitudes in CeA of alcohol-dependent rats. Notably, gabapentin infused into the CeA reverses dependence-induced increases in operant alcohol responding, but tends to increase alcohol drinking by nondependent rats (Roberto et al. 2008).

ALCOHOL AND GLUTAMATERGIC TRANSMISSION IN AMYGDALA

Glutamate, the major excitatory neurotransmitter has long been implicated in the reinforcing actions of alcohol. Glutamate receptors include three major classes of ionotropic receptors (iGluRs), with varying ratios of selectivity for Na^+, K^+, and Ca^{2+}. The iGluRs include AMPA (AMPARs), NMDA (NMDARs), and kainate receptors (KARs). Additionally, there are various subclasses of metabotropic glutamate receptors (mGluRs) that are G-protein-coupled receptors (GPCRs).

In contrast to its potentiating effects on GABA systems, alcohol generally inhibits glutamate neurotransmission in the brain (Lovinger and Roberto 2011). Dysregulation of glutamate systems may contribute to hyperexcitability and craving associated with alcohol withdrawal (Pulvirenti and Diana 2001). In the CeA of alcohol-preferring (P) rats, long-term consumption of high quantities of alcohol increases expression of mGluRs, NMDA receptor subunits, and a scaffolding protein regulating expression of these receptors in the cell membrane (Obara et al. 2009). Group II mGluRs also may block stress- and cue-induced reinstatement of alcohol-seeking behavior via neuronal activation in CeA or BNST (Zhao et al. 2006). Excitatory transmission in the CeA may also mediate some of the aversive aspects of withdrawal from abused drugs [e.g., morphine (Watanabe et al. 2002)].

Acamprosate, approved for treatment of alcoholic patients, modulates glutamate transmission via actions at NMDARs and/or mGluRs (Berton et al. 1998; Blednov and Harris 2008; Mann et al. 2008). Interestingly, acamprosate dampens the increased glutamate levels in abstinent alcoholics measured by magnetic resonance spectroscopy (Umhau et al. 2010) and reduces excessive alcohol drinking in alcoholics, presumably by reducing craving and negative affect (for a review, see Littleton 2007). AMPARs may be important in regulating relapse-like behaviors without playing a central role in alcohol consumption per se (Sanchis-Segura et al. 2006).

ACUTE ALCOHOL EFFECTS ON GLUTAMATERGIC TRANSMISSION IN CeA

We showed that acute alcohol (5–66 mM) decreases excitatory postsynaptic potentials (EPSPs) and currents (EPSCs) in the CeA, and that these effects are mediated by both NMDAR and non-NMDAR mechanisms (Roberto et al. 2004b). In contrast to alcohol effects on GABA release, the majority of studies indicate that acute alcohol either has no effect on or inhibits glutamate release (for a review, see Siggins et al. 2005) and inhibits NMDAR, AMPAR, and KAR function in some neuron types (for a review, see Lovinger and Roberto 2011).

CHRONIC ALCOHOL EFFECTS ON GLUTAMATERGIC TRANSMISSION IN CeA

Chronic alcohol exposure produces neuroadaptation in glutamatergic synaptic transmission. For example, acute alcohol decreases NMDAR-mediated EPSPs and EPSCs in the CeA of alcohol-dependent rats more than in alcohol-naïve controls. With local NMDA application, acute alcohol inhibits NMDA currents more in slices from alcohol-dependent rats, suggesting that alcohol dependence sensitizes NMDARs to alcohol (Roberto et al. 2004b, 2006). NMDARs containing the NR2B subunit are most sensitive to chronic alcohol exposure (Floyd et al. 2003; Carpenter-Hyland et al. 2004; Roberto et al. 2004b; Kash et al. 2009). Chronic alcohol increases NR2B mRNA and/or protein levels (Roberto et al. 2006; Kash et al. 2009) in the CeA and BNST, but not in other brain regions (Cebers et al. 1999; Floyd et al. 2003; Lack et al. 2005). It is not yet clear if increased NR2B subunit expression is *the* major driving force behind alcohol-induced increases in NMDAR function, or what molecular mechanisms underlie these subunit changes.

Microdialysis experiments in the amygdala show increases in glutamate release following chronic alcohol exposure (at 2–8 h withdrawal in CeA: Roberto et al. 2004b; at 24 h withdrawal in BLA: Lack et al. 2007). Our laboratory found that chronic alcohol exposure unmasks the ability of acute alcohol to increase presynaptic glutamate release in CeA (Roberto et al. 2004b), and that this effect persists 2 weeks into abstinence (Roberto et al. 2006). Collectively, these data suggest that multiple factors contribute to increased extracellular glutamate levels and increased glutamatergic transmission following chronic alcohol exposure and withdrawal.

CENTRAL AMYGDALA NEUROPEPTIDES AND ALCOHOL DEPENDENCE

Neuropeptides play prominent roles in regulating anxiety-like and alcohol-drinking behaviors in subjects that are either alcohol dependent, genetically vulnerable to developing excessive drinking (e.g., via selective breeding), repeatedly cycled through periods of alcohol withdrawal, and/or innately anxious. The CeA contains high concentrations of prostress (e.g., CRF) and antistress (e.g., NPY and nociceptin) neuropeptides, and many effects of these neuropeptides on anxiety- and alcohol-related behaviors have been localized to the CeA. Here we review the actions of a few neuropeptides on inhibitory transmission in the CeA, with focus on the CRF system that is heavily recruited during the transition from casual alcohol use to dependence (Koob 2010).

The CeA is the major output center of the amygdala, and about 95% of CeA neurons are medium spiny GABAergic neurons (McDonald 1982). The CeA is not a homogeneous neuroanatomical structure, and can be subdivided into lateral (CeL) and medial (CeM) aspects that differ in neuropeptide content, origin of afferents, and target sites of efferent projections (for a review, see Pitkanen et al. 2000). The CeL contains a much higher density of neuropeptides (e.g., dynorphin, CRF [Veening et al. 1984; Cassell et al. 1986; Shimada et al. 1989]) than the CeM, receives input from cortex and thalamus, and projects to the substantia innominata. In contrast, the CeM receives prominent inputs from other amygdaloid nuclei (especially glutamatergic afferents from BLA), and projects to effector regions such as hypothalamus and brain stem nuclei (Krettek and Price 1978). Both the CeL and CeM project to the BNST (Sun et al. 1991).

CRF AND ALCOHOL-RELATED BEHAVIOR

CRF plays a central role in arousal and hormonal, sympathetic, and behavioral responses to stress. The CeA, BNST, and BLA contain abundant CRF neurons and receptors (De Souza et al. 1984; Sakanaka et al. 1986). Hyperfunction of CRF systems in the CeA, BLA, and BNST produce increases in anxiety-like behavior (Sajdyk et al. 1999; Rainnie et al. 2004; Lee et al. 2008). Extracellular CRF levels in CeA are elevated following exposure to stress and development of alcohol dependence (Merlo Pich et al. 1995; Zorrilla et al. 2001), and alcohol withdrawal increases CRF synthesis and release in CeA

(Funk et al. 2006; Sommer et al. 2008; Roberto et al. 2010b). Likewise, alcohol withdrawal increases extracellular CRF in BNST (Olive et al. 2002), and these increases are normalized by alcohol consumption. CRFR antagonists suppress dependence-induced increases in alcohol drinking during acute withdrawal and protracted abstinence (Valdez et al. 2002) and reverse increases in stress-induced anxiety during protracted abstinence (Valdez et al. 2003). CRF repeatedly administered into the CeA, BLA, or dorsal BNST exaggerates alcohol withdrawal-induced increases in anxiety-like behavior via CRF_1Rs (Huang et al. 2010). Conversely, antagonism of CRFRs in the CeA blunts the increases in anxiety-like behavior in rats during withdrawal from chronic high-dose alcohol exposure (Rassnick et al. 1993).

CRF_1R antagonists block the anxiogenic effects of many stressors (Arborelius et al. 2000; Zorrilla and Koob 2004). CRF_1R antagonists also block increases in alcohol self-administration elicited by stressors and withdrawal (Hansson et al. 2006; Funk et al. 2007; Gehlert et al. 2007; Marinelli et al. 2007; Lowery et al. 2008). Chronic treatment with a CRF_1R antagonist abolishes dependence-induced escalation of drinking in rats chronically exposed to high doses of alcohol (Roberto et al. 2010b). Likewise, (1) stressors and alcohol withdrawal increase CRF_1R expression in limbic brain regions (Aguilar-Valles et al. 2005; Sommer et al. 2008); (2) rats bred for high alcohol preference show increased anxiety-like behavior and CRF_1R levels (Ciccocioppo et al. 2006); and (3) CRF_1R knockout mice show decreased anxiety-like behavior (Muller et al. 2003) and decreased drinking following withdrawal from chronic high-dose alcohol (Chu et al. 2007).

CRF EFFECTS ON SYNAPTIC TRANSMISSION IN CeA

We showed that CRF robustly increases GABAergic transmission in CeA of rats (Roberto et al. 2010b) and mice (Nie et al. 2004, 2009). CRF increases, and CRF_1R antagonists decrease, presynaptic GABA release, suggesting tonic facilitation of GABA release by endogenous CRF. CRF_1R antagonists and CRF_1R knockouts also block the alcohol-induced augmentation of GABAergic transmission in CeA. Both CRF- and alcohol-induced facilitation of GABAergic transmission in CeA require the PKCε signaling pathway (Bajo et al. 2008). Alcohol-dependent rats show increased sensitivity to the effects of CRF and CRF_1R antagonists on GABA release in CeA, suggesting up-regulation of the CRF-CRF_1R system. These findings are further supported by increased CRF and CRF_1R messenger RNA (mRNA) levels seen in the CeA of alcohol-dependent rats, and by reversal of dependence-induced elevations in amygdalar GABA dialysate by a CRF_1R antagonist (Roberto et al. 2010b). CRF also increases GABAergic transmission in the BNST, likely via actions at postsynaptic CRF_1Rs (Kash and Winder 2006; Francesconi et al. 2009).

NOCICEPTIN/ORPHANIN FQ AND ALCOHOL-RELATED BEHAVIOR

Nociceptin is an opioid-like peptide (Meunier et al. 1995; Reinscheid et al. 1995; Meunier 1997) that acts at opioid-like (NOP) receptors, although it does not bind to opioid receptors and opioids do not bind to NOP receptors (NOPRs). Nociceptin is abundantly expressed in the CeA and BNST (Neal et al. 1999) and is described as a functional CRF antagonist (Ciccocioppo et al. 2003). Nociceptin and other NOPR agonists have an anxiolytic-like profile in animal studies (Jenck et al. 1997, 2000). Nociceptin knockout mice show increased anxiety-like behavior (Koster et al. 1999) and are more sensitive to social stress (Ouagazzal et al. 2003).

Nociceptin suppresses alcohol drinking and prevents relapse-like behavior in rats (Ciccocioppo et al. 2004; Kuzmin et al. 2007). Nociceptin blocks reinstatement of alcohol-seeking behavior by cues predictive of alcohol availability and footshock stress (Martin-Fardon et al. 2000; Ciccocioppo et al. 2004), a behavior mediated by brain CRF systems (Ciccocioppo et al. 2004). Generally, rats bred for high alcohol intake show increased sensitivity to the suppressive effects of nociceptin on drinking and related behaviors (Economidou et al. 2008,

2011). Human alcoholics express decreased levels of mRNA for NOPR1 in the CeA (Kuzmin et al. 2007), suggesting that the CeA is a critical site for a role of nociceptin in alcoholism.

NOCICEPTIN AND SYNAPTIC TRANSMISSION IN CeA

We found that nociceptin dose-dependently and reversibly reduced $GABA_A$R-mediated IPSCs in CeA (Roberto and Siggins 2006). Nociceptin increased PPF of evoked IPSCs and decreased the frequency of mIPSCs in CeA, suggesting decreased presynaptic GABA release. Notably, nociceptin both prevented (when applied before alcohol) and totally reversed (applied during alcohol) acute alcohol-induced increases in evoked IPSC amplitudes and mIPSC frequencies and decreases in PPF, thus preempting the usual alcohol-induced increase in GABA release in CeA. Further, the ability of nociceptin to decrease GABAergic transmission in CeA is augmented following alcohol dependence, suggesting that the nociceptin system in the CeA adapts during chronic alcohol exposure (Roberto and Siggins 2006).

NEUROPEPTIDE Y (NPY) AND ALCOHOL-RELATED BEHAVIOR

The amygdala contains abundant NPY fibers and receptors (Allen et al. 1984; de Quidt and Emson 1986; Dumont et al. 1993; Gustafson et al. 1997). NPY anxiolytic effects may involve both the CeA (Heilig et al. 1993) and BLA (Sajdyk et al. 1999). Rats selectively bred for high alcohol preference express low levels of NPY and NPY mRNA in CeA that are restored by voluntary alcohol intake (Pandey et al. 2005). Alcohol-withdrawn rats show increases in anxiety-like behavior and decreased amygdalar NPY, possibly owing to changes in histone acetylation (Roy and Pandey 2002; Zhao et al. 2007; Pandey et al. 2008).

NPY microinjection into the CeA suppresses alcohol consumption in alcohol-dependent and abstinent P rats (Gilpin et al. 2008a,b), and also in rats showing innately high anxiety-like behavior (Primeaux et al. 2006). Both postsynaptic Y_1 and presynaptic Y_2 receptors (Y_1Rs and Y_2Rs) are implicated in the effects of NPY on anxiety-like behavior and alcohol consumption. Early studies suggested roles for amygdalar Y_1Rs (Heilig et al. 1993) and Y_2Rs (Sajdyk et al. 2002) in anxiety-like behavior. Mouse studies indicate that Y_1Rs mediate the suppressive effects of NPY on alcohol drinking (Thiele et al. 2002; Sparta et al. 2004; Eva et al. 2006). Likewise, acute stress and alcohol withdrawal increase amygdalar Y_1R expression in rodents (Aguilar-Valles et al. 2005; Eva et al. 2006; Sommer et al. 2008).

NPY AND SYNAPTIC TRANSMISSION IN CeA

Our lab found that NPY in CeA prevents acute alcohol-induced increases in evoked IPSPs and mIPSC frequency (Gilpin et al. 2011) and PPF decreases, suggesting that NPY effects arise from decreased presynaptic GABA release. Tests with antagonists confirm the presynaptic site of action and suggest that NPY blocks alcohol effects via actions at presynaptic Y_2Rs. NPY also normalizes alcohol dependence-induced increases in GABA release in CeA, suggesting that chronic exposure causes neuroadaptations in NPY systems that affect inhibitory transmission here. These results concur with findings that NPY modulates GABA release in BNST via activation of presynaptic Y_2Rs (Kash and Winder 2006) and suggest that Y_2Rs function as autoreceptors regulating NPY release (Chen et al. 1997), and also as heteroceptors regulating release of other neurotransmitters (Greber et al. 1994). NPY actions at postsynaptic Y_1Rs appear to function as a "brake" on presynaptic Y_2R-mediated reductions in GABA release, a result that may explain previous findings that intra-amygdala infusion of a Y_1R antagonist decreases alcohol drinking in rats (Schroeder et al. 2003). These combined results support the hypothesis that NPY blocks stress-induced reinstatement of alcohol-seeking behavior via activation of inhibitory neurons in CeA (Cippitelli et al. 2010).

DISINHIBITION MODEL OF CeA OUTPUT

As noted above, most neurons in the CeA are GABAergic inhibitory projection neurons or

interneurons that cotransmit GABA and one or more neuropeptides. Peptides that promote anxiety-like behavior and alcohol self-administration (e.g., CRF) generally increase, whereas peptides that reduce anxiety-like behavior and alcohol self-administration (e.g., nociceptin, NPY) decrease GABAergic transmission in CeA. In our slice preparation, we stimulate and record locally in the medial portion of the CeA, and recordings of GABAergic transmission reflect the activity of inhibitory interneurons or projection neurons (via collaterals) within CeA. Therefore, increases in GABAergic transmission within CeA following application of acute alcohol or CRF will inhibit the activity of GABAergic neurons projecting out of CeA. Conversely, decreases in GABAergic transmission in CeA neurons (e.g., following nociceptin or NPY application) will reduce inhibition of GABAergic neurons projecting out of CeA, thereby facilitating release of GABA in downstream targets (e.g., BNST, periaqueductal gray). Thus, increases or decreases in inhibitory output from the CeA to downstream effector regions may decrease or increase anxiety-like behavior, respectively (Pare et al. 2004; Davis et al. 2010; Tye et al. 2011). Furthermore, alcohol markedly affects excitatory transmission in CeA, particularly via NMDARs (Roberto et al. 2004b, 2006), lending at least partial buffering of alcohol effects on inhibitory transmission. Finally, alcohol may alter the release of local opioids (Lam et al. 2008), endocannabinoids (Roberto et al. 2010a), and/or galanin (Bajo et al. 2011) in CeA that in turn may increase GABA-mediated inhibition of downstream target areas. Other neuropeptides (e.g., substance P, vasopressin) are also likely to regulate the synaptic transmission within CeA.

CONCLUSIONS

The data summarized here support the idea that the CeA is a critical locus of neuroadaptation during the transition to alcohol dependence. Alcohol has strong and persistent effects, particularly on inhibitory transmission, in the CeA of alcohol-dependent animals. Neuropeptides present at high levels in the CeA profoundly alter inhibitory transmission, and potentially also excitatory transmission. The ability of these neuropeptides to affect neurotransmission in the CeA, either alone or in combination with alcohol, is often dysregulated in alcohol dependence. Although manipulation of many of these peptides affects alcohol drinking in alcohol-dependent but not nondependent animals, it is unsurprising that these neuropeptides affect basal neurotransmission in CeA of alcohol-naïve animals, especially because all these peptides when microinjected into CeA modulate anxiety-related behavior independent of alcohol exposure history. This point also enhances our understanding of why *these* neuropeptide systems are recruited and/or up-regulated during the transition to alcohol dependence, a dynamic disease defined largely by a negative emotional state in the absence of the drug. Finally, our electrophysiological data suggest that synaptic transmission and the special neuronal circuitry in the CeA may be an important point of convergence for the neuroadaptations that occur during the transition to alcohol dependence. Our understanding of this pivotal system as a "bellwether" target for therapeutic testing for anxiety and alcohol use disorders may be reliably predicted by drug effects on synaptic transmission in the CeA. Thus, we predict that most drugs that decrease GABAergic transmission in CeA neurons will be logical candidates for treatment of anxiety and/or alcohol use disorders.

ACKNOWLEDGMENTS

This work is supported by the National Institute of Alcohol Abuse and Alcoholism grants AA015566, AA06420, AA016985, AA017447, AA018400, and AA13498. This is manuscript number 21515 from The Scripps Research Institute.

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