Archival Report

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Extended Amygdala to Ventral Tegmental Area Corticotropin-Releasing Factor Circuit Controls Binge Ethanol Intake

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ABSTRACT

BACKGROUND: Corticotropin-releasing factor (CRF) signaling at the CRF_1 receptor (CRF_1R) in the ventral tegmental area (VTA) can modulate ethanol consumption in rodents. However, the effects of binge-like ethanol drinking on this system have not been thoroughly characterized, and little is known about the role of CRF_2R or the CRF neurocircuitry involved.

METHODS: The effects of binge-like ethanol consumption on the VTA CRF system were assessed following drinking-in-the-dark procedures. Intra-VTA infusions of selective CRF_1R and/or CRF_2R compounds were employed to assess the contributions of these receptors in modulating binge-like ethanol consumption ($n = 89$). To determine the potential role of CRF projections from the bed nucleus of the stria terminalis (BNST) to the VTA, CRF neurons in this circuit were chemogenetically inhibited ($n = 32$). Binge-induced changes in VTA CRF system protein and messenger RNA were also assessed ($n = 58$).

RESULTS: Intra-VTA antagonism of CRF_1R and activation of CRF_2R resulted in decreased ethanol intake, which was eliminated by simultaneous blockade of both receptors. Chemogenetic inhibition of local CRF neurons in the VTA did not alter binge-like ethanol drinking, but inhibition of VTA-projecting CRF neurons from the BNST significantly reduced intake.

CONCLUSIONS: We provide novel evidence that 1) blunted binge-like ethanol consumption stemming from CRF_1R blockade requires intact CRF_2R signaling, and CRF_2R activation reduces binge-like drinking; 2) inhibiting VTA-projecting BNST CRF neurons attenuates binge-like drinking; and 3) binge-like ethanol drinking alters protein and messenger RNA associated with the VTA-CRF system. These data suggest that ethanol-induced activation of BNST-to-VTA CRF projections is critical in driving binge-like ethanol intake.

Keywords: Binge drinking, Corticotropin-releasing factor, Drinking in the dark, Ethanol, Extended amygdala, Ventral tegmental area

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The transition from moderate controlled drinking to ethanol dependence is usually accompanied by intermittent bouts of binge consumption of ethanol, culminating in heavy, uncontrolled ethanol consumption. The National Institute of Alcohol Abuse and Alcoholism defines a binge as a pattern of drinking characterized by consuming enough ethanol to rapidly achieve blood ethanol concentrations (BECs) >80 mg/dL (0.08%) (1). Because this pattern of drinking often leads to the development of ethanol dependence (2,3), examining the systems that are recruited or dysregulated during repeated episodes of binge ethanol drinking provides an opportunity to understand the mechanisms involved and identify potential therapeutic targets to prevent the transition to ethanol dependence.

One system that has been strongly implicated in alcohol use disorders is corticotropin-releasing factor (CRF) and its receptors (4). A 41–amino acid peptide, CRF is canonically involved in the stress response, exerting its effects through two G protein–coupled receptors, the CRF1 receptor (CRF_1R) and CRF_2R (5). CRF has a higher affinity for CRF_1R than CRF_2R; higher concentrations of CRF are necessary to activate both receptor subtypes (6). Evidence shows that CRF and CRF_1R modulate dependence-induced ethanol intake (7–11), suggesting that CRF_1R signaling becomes dysregulated with dependence promoting drinking. However, evidence suggests that CRF signaling is also recruited during binge-like ethanol drinking before dependence (12,13). Lowery-Gionta *et al.* (13) showed that CRF protein levels were significantly increased in the central amygdala (CeA) after binge-like ethanol consumption, and blockade of CRF_1R in the CeA blunted binge-like ethanol consumption. This finding is analogous to the observation that CeA-infused CRF_1R antagonists protect against dependence-induced escalation of ethanol consumption (4,8). Lowery-Gionta *et al.* (13) reported that CRF protein levels in the ventral tegmental area (VTA) were also significantly increased after binge-like ethanol drinking. Consistently, more recent data have demonstrated

SEE COMMENTARY ON PAGE 905

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that intra-VTA CRF_1R antagonists can reduce escalated ethanol consumption in chronically drinking mice (14) and blunt binge-like ethanol drinking (15). Additionally, selective inhibition of CRF neurons in the bed nucleus of the stria terminalis (BNST), which projects to the VTA, also reduces binge-like ethanol consumption (16). Despite evidence that central CRF_2R agonists also reduce ethanol consumption (9,12), the role of VTA CRF_2R activation, or the interaction between CRF_1R and CRF_2R, has not been explored.

The function of CRF signaling in the VTA is of particular interest because of the role the VTA plays in regulating the reinforcing properties of drugs and ethanol (17–20), yet the mechanisms by which CRF signaling in the VTA modulates binge-like ethanol intake are still largely unknown. In this study, we show that 1) blunted binge-like ethanol drinking stemming from CRF_1R blockade requires intact CRF_2R signaling, and CRF_2R activation reduces binge-like drinking; 2) silencing VTA-projecting CRF neurons originating in the BNST attenuates binge-like drinking; and 3) a history of binge-like ethanol drinking alters protein and messenger RNA (mRNA) expression of candidate targets of the VTA CRF system.

METHODS AND MATERIALS

Animals

Male C57BL/6J mice (Jackson Laboratories, Bar Harbor, ME) 8–10 weeks old at the start of the experiment were used except for the in vivo chemogenetic experiment, in which male CRF-ires-Cre (CRF-Cre) mice (positive for the expression of Cre recombinase under the CRF promoter as determined by standard polymerase chain reaction [PCR] genotyping protocols) at least 10 weeks old were used. CRF-Cre mice were generated as previously described in detail (16,21).

Drugs and Solutions

Details regarding drugs, doses, and solutions are described in detail in Supplemental Methods and Materials.

Drinking in the Dark Procedure

Binge-like ethanol consumption was induced using a standard 4-day drinking in the dark (DID) protocol (22,23). Approximately 3 hours into the dark cycle, home cage water bottles were removed, and animals were given access to a bottle containing 20% ethanol for 2 hours on days 1–3 (training days) and for 2–4 hours on day 4 (binge test [BT]). Immediately after the BT, ~30 μL of tail blood was collected by nicking the lateral tail vein, and BECs were determined using the Analox Analyzer (Analox Instruments, Lunenburg, MA). See Supplemental Methods and Materials for additional details.

In Vivo Pharmacology

Intra-VTA CRF_1R Antagonism. An initial cohort of C57BL/6J mice (n = 20) experienced one cycle of ethanol DID to establish baseline drinking and were subsequently implanted with bilateral cannulas aimed at the VTA. After 1 week of recovery, mice experienced a second cycle of DID and were rank-ordered based on average consumption on days 1–3 and assigned to receive an intra-VTA microinfusion of either antalarmin or vehicle ~1 hour before ethanol access on the BT, such that ethanol consumption was equated between treatment conditions. Because a number of animals in this experiment lost cannulas, mice experienced a third cycle of DID and were assigned to receive either vehicle or antalarmin in a Latin square design (final n = 10/treatment after excluding cannula loss [n = 4] and placements outside of the VTA [n = 6]). These mice then experienced two cycles of DID with sucrose in a Latin square design (final n = 10/treatment).

Intra-VTA CRF_2R Activation. To examine the effects of CRF_2R activation in the VTA on binge ethanol consumption, a second cohort of mice (n = 20) experienced one cycle of ethanol DID and were subsequently implanted with bilateral cannulas aimed at the VTA. After a second cycle of DID and ~1 hour before ethanol access on the BT, the mice received an infusion of either urocortin III (Ucn3) or vehicle (n = 9 or 10 per treatment). A sufficient number of mice retained their cannulas (n = 19), and a Latin square design was unnecessary. These mice then experienced one cycle of sucrose DID and received the opposite treatment they received during the ethanol DID.

Anatomic Control Site for CRF_1R Antagonism and CRF_2R Activation. To ensure that the effects of CRF_1R antagonism and CRF_2R activation were specific to the VTA and not due to drug traveling up the injector path, a third cohort of animals (n = 16) experienced one cycle of ethanol DID and were then implanted with cannulas aimed at a region ~2 mm dorsal to the VTA. During a second cycle of DID, mice were assigned to receive either antalarmin or vehicle (n = 8/treatment). On the third cycle of DID, mice that previously received drug now received vehicle, and mice that previously received vehicle now received Ucn3 (n = 8/treatment).

Simultaneous CRF_1R and CRF_2R Antagonism. To examine the relative contribution of each receptor to the reduction in binge-like ethanol consumption, a fourth cohort of mice (n = 44) experienced one cycle of ethanol DID and were subsequently implanted with bilateral cannulas aimed at the VTA. After recovery, the mice experienced a second cycle of DID and were assigned to receive vehicle (n = 11), the CRF_1R antagonist NBI 35965 (n = 11), the CRF_2R antagonist K 41498 (n = 11), or both NBI 35965 and K 41498 simultaneously (n = 11).

In Vivo Chemogenetic Manipulation

Inhibition of BNST to VTA Projecting CRF Neurons. Given previous evidence that $G_{i/o}$-coupled designer receptors exclusively activated by designer drugs (DREADDs) (24) cause functional inhibition of CRF neurons when transfected into CRF-Cre mice (16), we decided to examine the role of CRF neurons projecting from the BNST to the VTA. CRF-Cre mice (n = 16) experienced one cycle of ethanol DID before surgery. Mice were then injected with either a Cre-dependent control vector (AAV8-hSyn-DIO-mCherry, n = 8) or the Cre-dependent $G_{i/o}$-coupled DREADD vector