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β-endorphin regulates alcohol consumption induced by exercise restriction in female mice

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ABSTRACT

Animal models have long been used to study the mechanisms underlying the complex association between alcohol and stress. Female mice prevented from running on a home–cage activity wheel increase voluntary ethanol consumption. β-endorphin is an endogenous opioid involved in negatively regulating the stress response and has also been implicated in the risk for excessive drinking. The present study investigates the role of β-endorphin in moderating free-choice consumption of ethanol in response to a blocked activity wheel. Female, transgenic mice with varying levels of the opioid peptide were given daily 2-h access to 20% ethanol with rotations on a running wheel blocked on alternate days. Subjects with low β-endorphin exhibited enhanced stress sensitivity by self-administering larger quantities of ethanol on days when wheel running was prevented. β-endorphin levels did not influence voluntary activity on the running wheel. There were genotypic differences in plasma corticosterone levels as well as corticotropin-releasing hormone mRNA content in multiple brain regions associated with the stress response in these free drinking and running subjects. Susceptibility to stress is enhanced in female mice with low levels of β-endorphin, and better understanding of the role for this opioid in mitigating the response to stressors may aid in the development of interventions and treatments for excessive use of alcohol in women.

Introduction

Alcohol is a physiological stressor, potently activating the neuroendocrine stress response, yet people often consume the drug as a way to cope with stress. In part because both stress and alcohol impact and recruit a multitude of factors, the mechanisms underlying their relationship remain largely unclear (Phillips, Reed, & Pastor, 2015; Stephens & Wand, 2012). Attempts to better understand the complex interactions between stress and ethanol have frequently employed animal models (Crabbe, 2014), and these strategies have helped to identify some of the specific mechanisms contributing to aspects of the paradoxical relationship, especially as they pertain to the dependent state (Becker, Lopez, & Doremus-Fitzwater, 2011; Crabbe, Phillips, & Belknap, 2010). Our lab has employed intermittent interruptions of access to a running wheel as a model of stress (Ehringer, Hoft, & Zunhammer, 2009; Piza-Palma et al., 2014). We have argued that blocking access to a running wheel in the home cage induces frustration stress, and that this manipulation may be relevant to the human condition where stressors often involve loss of something desired, such as a loved one, health, or job (Thoits, 2010).

Individual differences in drug use and abuse as well as stress susceptibility are impacted by a vast number of factors. These factors include environmental challenges, genetic background, family history, and gender. Restricting rotations of an appetitive running wheel results in significant increases in voluntary ethanol consumption in female, but not male, C57BL/6J mice (Piza-Palma et al., 2014). Because women are generally more sensitive to stress (Burk et al., 2011; Randall et al., 1999; Young-Wolff, Kendler, & Prescott, 2012) and prone to disproportionately escalating their use and abuse of the drug compared to men (Becker et al., 2005; Greenfield, Back, Lawson, & Brady, 2010; Keyes, Grant, & Hasin, 2008), this sex-dependent effect may be a useful tool in addressing the general shortage of research on sex-dependent factors (Beery & Zucker, 2011) and may help shed light on the general relationship between stress and alcohol in non-dependent subjects.

Stress is a multifaceted adaptation to environmental perturbation that can evoke a wide range of physiological and behavioral changes. One such consequence is the increased synthesis and

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secretion of corticotropin-releasing hormone (CRH), also implicated as a key player in chronic ethanol exposure and dependence (Phillips et al., 2015). In response to stressors (including ethanol), CRH stimulates transcription of the proopiomelanocortin gene, which produces precursors for adrenocorticotropin hormone (ACTH) and β-endorphin. While ACTH is carried in the blood to help coordinate the peripheral stress response, β-endorphin, an endogenous opioid peptide, modulates the hypothalamic-pituitary-adrenal (HPA) axis (Charmandari, Tsigos, & Chrousos, 2005; Pechnick, 1993) by inhibiting secretion of CRH (Buckingham, 1986; Plotsky, 1991; Sarkar, Kuhn, Marano, Chen, & Boyadjieva, 2007). β-endorphin also contributes to behavioral stress responses in a number of ways (Amir, 1982; Ribeiro, Kennedy, Smith, Stohler, & Zubieta, 2005; Yamada & Nabeshima, 1995). Our lab has shown that mice lacking this peptide exhibit exaggerated behavioral responses to stressors (Barfield, Moser, Hand, & Grisel, 2013; Barfield et al., 2010; Grisel, Bartels, Allen, & Turgeon, 2008; Grisel et al., 1999). Moreover, a clinical correlation has been established between heritable levels of β-endorphin and risk for excessive drinking (Froehlich, Harts, Lumeng, & Li, 1990; Gianoulakis, 2009; Wand, Mangold, El Deiry, McCaul, & Hoover, 1998). This body of research supports the contention that β-endorphin modulates the relationship between stress and alcohol.

Using the transgenic model for β-endorphin deficit developed by Rubinstein and colleagues in 1996, we have shown that heterozygous mice (βE-HT), with reduced levels of the opioid peptide, consume slightly but significantly more ethanol in standard two-bottle choice experiments than either wild-type controls or mice entirely lacking the opioid (βE-KO) (Grisel et al., 1999; Williams, Holloway, Karwan, Allen, & Grisel, 2007). We have previously argued that βE-HT mice find ethanol especially rewarding since the drug can stimulate production of the peptide and ameliorate a deficient state. Because β-endorphin deficiency also results in increased stress sensitivity, it is possible that the tendency to self-medicate would be even higher under stressful conditions.

The purpose of our study was to investigate voluntary drinking in mice with varying levels of β-endorphin in the context of external stress from a blocked running wheel. In order to begin exploring the effects of constitutive β-endorphin deficiency on endocrine responses to stress, we also evaluated plasma ACTH, corticosterone (CORT), and mRNA for the CRH peptide and its type 1 receptor (CRH-R1) in brain areas implicated in the stress response, including the ventral hippocampus (VH), amygdala and bed nucleus stria terminalis (BNST), and dorsomedial prefrontal cortex (dmPFC) (Silberman & Winder, 2013; Stamatakis et al., 2014). A modified drinking-in-the-dark paradigm was employed with locked running wheels acting as an external stressor in female C57BL/6J, βE-HT, and βE-KO mice (Ehringer et al., 2009; Piza-Palma et al., 2014). We hypothesized the finding of increased drinking in β-endorphin-deficient mice in response to stress, and speculated that the differences in behavioral sensitivity to stress would be reflected in heightened ACTH and/or CORT levels and alterations in *Crh* and/or *Crh-R1*.

Methods

Subjects

The β-endorphin-deficient model was developed about 20 years ago in the laboratory of Malcolm Low (Rubinstein et al., 1996) by insertion of a premature stop codon into the *Pomc* gene. The gene mutation has been fully backcrossed to the C57BL/6J strain (>20 generations). Homozygotes (KO) cannot synthesize β-E, though all other products of the POMC protein show normal expression. Opioid receptor expression also remains unchanged (Rubinstein et al., 1996). The model has been used in studies of metabolism, as KO males (but not females, which were used here) show an altered growth curve resulting in increased body mass and white fat (Low, Hayward, Appleyard, & Rubinstein, 2003). We previously suggested hypersensitivity to stress in β-E deficient mice (e.g., Barfield et al., 2013) but no overt alterations in the HPA axis have been reported, and homozygous mutant mice appear otherwise normal in terms of development and behavior. HT mice produce 50% of B6 levels of β-E.

Thirty-six adult naïve female mice between the ages of 55 and 81 days at the start of the experiment were used. Mice for these studies were bred in-house from stock purchased from Jackson Laboratories (Bar Harbor, ME). βE-HT mice were bred from βE-KO males and B6 females; others were bred under identical conditions from genotype-matched pairs. Subjects included 13 B6, 13 βE-HT, and 10 βE-KO mice. Mice were weaned at 21 days and group-housed by sex and genotype in Plexiglas® cages filled with corn cob bedding in a colony with a 12-h reverse light:dark cycle (lights off at 0930) maintained at 21 ± 2 °C. Subjects were given free access to standard mouse chow and tap water at all times before and during the study.

During the experimental period, subjects were moved to an experimental room across the hall from the colony that was maintained with the same temperature and light conditions. However, during a 4-day habituation period and throughout the 10-day experimental period, subjects were housed individually in TSE Phenomaster Plexiglas® cages that contained a running wheel (11 cm in diameter; TSE Systems, Bad Homburg, Germany) in addition to *ad libitum* access to food and water and limited access to ethanol (see below). The corn cob bedding was changed once during the experiment, between habituation and the beginning of the experimental period.

Drinking procedure

A modified drinking-in-the-dark procedure (Ehringer et al., 2009; Rhodes et al., 2007) was used throughout this study. Thus, from the time subjects were individually housed in the experimental room, they were allowed access to 20% ethanol (v:v in tap water) for 2 h each day, beginning 3 h into their dark cycle, always with food and tap water freely available. Ethanol presentation was switched every 2 days to prevent the development of a side preference. During the 10-day experimental period, wheel rotations were limited every other day. On unlocked days (1, 3, 5, 7, and 9) the wheel freely rotated for active animals as during habituation, but on locked days (2, 4, 6, 8, and 10) a brake was remotely engaged so that the wheel could not rotate beginning 1 h before, and continuing throughout the ethanol-access period. The TSE PhenoMaster program was intended as our method for collecting both drinking data and running data, but the program generated unreliable and incorrect fluid consumption data. Therefore, the TSE PhenoMaster program measured only running data, and drinking was assessed manually by reading gradations on a 13-mL tube with a ball-bearing sipper. Each day we calculated the dose administered by each mouse (g ethanol/kg of body weight) as well as ethanol preference (the percentage of total fluid consumed that was from the ethanol-filled tube) during the 2 h of ethanol access. Twelve mice could be tested at a time in our facility, so subjects were tested in three runs, with efforts to counterbalance genotype within and between runs, and with individuals randomly assigned to cages in the testing room.

Blood ethanol content (BEC) analysis

Immediately following the final experimental manipulation on Day 10 (locked running wheel for 1 h before and 2 h during