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Naltrexone alters alcohol self-administration behaviors and hypothalamic-pituitary-adrenal axis activity in a sex-dependent manner in rats

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ABSTRACT

Background: The mu-opioid antagonist, naltrexone (NTX), is a FDA-approved treatment for alcohol use disorder (AUD); however, the data on whether it differentially affects males vs. females are mixed. NTX increases hypothalamic-pituitary-adrenal (HPA) axis activity that associates with subjective responses to alcohol and craving in individuals with AUD. The present study tested for sex differences in the ability of NTX to decrease appetitive and consummatory behaviors in rats in operant alcohol self-administration. Because the opioid system and HPA axis are sexually dimorphic, we examined NTX's effect on adrenocorticotropic hormone (ACTH) and corticosterone (CORT) levels.

Methods: Male and female Sprague-Dawley rats (n's = 6–8) were trained to lever press for alcohol (10% v/v) under a fixed-ratio 2 schedule of reinforcement. NTX doses (0, 0.1–10 mg/kg) were assessed in tests conducted under a progressive ratio schedule of reinforcement. Separate groups of alcohol and water drinking rats (n's = 8) were used to assess NTX's (10 mg/kg) effects on HPA axis hormones.

Results: NTX decreased consummatory behaviors for alcohol in a dose-related manner, but not appetitive behaviors in males. In females, NTX decreased appetitive behaviors for alcohol in a dose-dependent manner, but only decreased consummatory behaviors at the highest (10 mg/kg) NTX dose. NTX increased ACTH levels in alcohol drinking females in diestrus, but not in other groups. However, NTX increased CORT levels for longer durations in alcohol drinking males relative to alcohol drinking females in diestrus.

Conclusions: Our findings suggest that NTX selectively reduces consummatory behaviors for alcohol in males and appetitive behaviors in females, while also showing differential sex effects on HPA hormones.

1. Introduction

According to the 2015 National Survey on Drug Use and Health, 15 million adults had a diagnosis of alcohol use disorder (AUD) with > 5 million of these cases occurring in females (SAMHSA, 2015). Although AUD is twice as likely to be diagnosed in males, females are more susceptible to negative health consequences associated with alcohol consumption. For example, females who consume alcohol have an increased risk of cirrhosis of the liver and hepatitis (Loft et al., 1987), breast cancer (Smith-Warner et al., 1998; Hamajima et al., 2002), cardiovascular disease (Urbano-Marquez et al., 1995), and brain damage (Mann et al., 2005). Increased susceptibility to these conditions may be due to underlying sex differences in alcohol metabolism. For instance, females have greater blood alcohol levels after drinking equivalent amounts of alcohol (Baraona et al., 2001; Frezza et al., 1990) and less alcohol metabolizing enzymes compared to males (Baraona et al., 2001). Given the detrimental effect of AUD in females, it is essential to have treatments that are effective in this vulnerable population.

Naltrexone (NTX) is a mu opioid antagonist that has shown modest efficacy in treating AUD. In human laboratory studies, NTX reduces alcohol's positive subjective effects (Ray and Hutchison, 2007; Drobes et al., 2004; Ray et al., 2009) and exacerbates negative subjective effects (King et al., 1997; Ray et al., 2009), thereby contributing to attenuation of alcohol self-administration. NTX prevents alcohol-induced dopamine release in the ventral tegmental area and nucleus accumbens (Koob and Le Moal, 2008; Kreek, 1996), key brain regions involved in reward processes. In addition, NTX's efficacy in treating AUD may also be due to its effects on dopamine-independent mechanisms. Acute alcohol consumption stimulates the hypothalamic-pituitary-adrenal (HPA) axis (Jenkins and Connolly, 1968), while chronic alcohol intake leads to allostatic changes that contribute to blunted HPA activity in several HPA functional tests (Wand and Dobs, 1991; Vescovi et al., 1997; Sorocco et al., 2006) and in response to alcohol consumption (Adinoff et al., 1990; Inder et al., 1995). Indeed, individuals with AUD often suffer from neuroendocrine tolerance (Adinoff et al., 1998;

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Adinoff et al., 2005), a condition that associates with an increased risk of early relapse in humans (Junghanns et al., 2003; Junghanns et al., 2005) and doubles alcohol consumption in both continuous- and limited-access two bottle choice paradigms in mice (Olive et al., 2003). NTX stimulates the HPA axis in individuals with AUD by preventing beta endorphin-induced inhibition of corticotrophin releasing factor in the paraventricular nucleus of the hypothalamus (Zhou and Kreek, 2014). NTX-induced stimulation of HPA activity negatively associates with alcohol craving and risk of relapse in individuals with AUD (Farren et al., 1999; Kiefer et al., 2006; O'Malley et al., 2002). Interestingly, in a sample of hazardous drinkers that do not meet clinical criteria for AUD, no association between NTX-induced elevations in cortisol levels and alcohol craving was found (Ray et al., 2009). Unfortunately, NTX's efficacy in females with AUD has not been thoroughly explored. Additionally, there is a sparse literature examining NTX's effects on HPA activity in alcohol drinking females.

The few studies examining NTX's efficacy in females with AUD have revealed inconsistent findings (Agabio et al., 2016; Canidate et al., 2017). For example, in humans, studies have found decreases, increases, and no change in NTX's efficacy in males compared to females (Garbutt et al., 2005; Kiefer et al., 2005; Greenfield et al., 2010; Baros et al., 2008; Pettinati et al., 2008). There are several differences across clinical studies. For instance, there are variations in drinking outcomes measured, duration of NTX treatment, and drinking history of participants, among others. Furthermore, males and females are often collapsed together in statistical analyses examining the relationship between NTX and the HPA axis in alcohol drinking humans. Thus, it remains unclear whether naltrexone stimulates HPA axis activity in females with AUD, and if so, whether cortisol associates with appetitive and/or consummatory behaviors for alcohol in females. Animal studies are beneficial to address these questions in that the environment can be strictly controlled and variables of interest can be selectively manipulated and precisely measured. Although many animal studies have supported NTX's efficacy at reducing alcohol consumption, few have included females, especially in studies using operant self-administration procedures. To our knowledge, no study has examined sex differences in NTX's effect on operant alcohol self-administration using an outbred rat strain. Lack of such information is concerning given evidence that the opioid system (Zubieta et al., 1999; Zubieta et al., 2002) and HPA axis (Kudielka and Kirschbaum, 2005; Kitay, 1963; Kitay, 1961) are sexually dimorphic. In addition, males and females of outbred rat strains differ in appetitive and consummatory responses for alcohol in operant procedures (Nieto and Kosten, 2017; Bertholomey et al., 2016). Appetitive and consummatory behaviors characterize distinct stages of the addiction cycle (Koob et al., 2009) and are regulated by separate neurobiological processes (Slawecki and Roth, 2003; Sharpe and Samson, 2001). In addition, appetitive behaviors can interrupt consumption (Breland and Breland, 1961); thus, it would be useful to determine the efficacy of AUD pharmacotherapies on both behaviors within the same operant session (Kosten and Meisch, 2013). Therefore, the purpose of this study is to examine sex differences in NTX effects on appetitive and consummatory behaviors during operant alcohol self-administration and HPA axis activity in alcohol drinking Sprague-Dawley rats.

2. Materials and methods

2.1. Animals

Adult (postnatal day 90–100) male (400–500 g) and female (200–250 g) Sprague-Dawley rats (Charles River, Wilmington, MA) were used in this study. Rats were single-housed in amber polysulfone cages and kept in a temperature- and humidity-controlled vivarium maintained on a 12:12 light/dark cycle (lights on at 7:00 AM). Rats were single-housed to more accurately measure alcohol consumption during overnight drinking in the dark (described below). Additionally, females used in Experiment 1 were free-cycling, while females in diestrus were used in Experiment 2. Animals were given ad libitum access to food and water except during fluid restriction as described below. The Institutional Animal Care and Use Committee at the University of Houston approved the experimental procedures in accordance with guidelines set forth in the "Guide for the Care and Use of Laboratory Animals 8th Edition".

2.2. Solution and drug preparations

Alcohol (ethyl alcohol, 190 Proof, USP grade, Koptec, King of Prussia, PA) was mixed with tap water to reach a concentration of 10% alcohol (v/v) solution. Naltrexone HCI (NTX; Sigma-Aldrich, St. Louis, MO) was dissolved in isotonic saline at a concentration of 1 mg/mL. NTX was administered subcutaneously (SC) at the following doses: 0 (isotonic saline), 0.1, 0.3, 1, 3, and 10 mg/kg immediately prior to the start of the test session. In the operant studies, each dose was tested twice per animal and the means of those two tests were used. Order of dose presentation was non-systematic and counterbalanced across animals.

2.3. Alcohol drinking in the dark schedule

Rats in the operant studies (Experiment 1) were subjected to a drinking in the dark (DID) schedule beginning two weeks before self-administration training and continued for the remainder of the experiment. Outbred rat strains do not readily self-administer unadulterated alcohol; thus, certain procedures have been developed to facilitate operant alcohol self-administration (Bell et al., 2017). A prior drinking history is one approach to encourage operant alcohol self-administration in outbred rats (Weiss, 2011). Thus, rats were kept on DID throughout Experiment 1 to maintain stable self-administration. Alcohol drinking rats used for the HPA axis hormone studies (Experiment 2) remained on DID schedule for 16 weeks (Table 1). DID was conducted as described previously in (Nieto and Kosten, 2017). Briefly, rats were given access to only 10% alcohol (v/v) for a 16 h period (5 pm to 9 am) with water available for 1 h during the mornings. Self-administration procedures and blood withdrawals began 3–6 h after the end of the DID period. Since rats advanced through the self-administration phases individually, only alcohol intake prior to NTX administration was analyzed. Alcohol intake and body weights were measured weekly throughout the entire course of the study. Alcohol intake was converted to g/kg to provide amount of alcohol consumed.

Table 1
Experimental groups and testing sequence.

Experiment	n	Sex	Procedure 1	Procedure 2	NTX dose	Procedure 3	Data
1	8	Female	DID (26 Weeks)	SA training	0–10 mg/kg; SC	PR testing	Fig. 2
	6	Male	DID (26 Weeks)	SA training	0–10 mg/kg; SC	PR testing	Fig. 2
2	8	Female	CON & DID (16 weeks)	Hormone assays	0 & 10 mg/kg; SC	–	Figs. 1, 3, & 4
	8	Male	CON & DID (16 Weeks)	Hormone assays	0 & 10 mg/kg; SC	–	Figs. 1, 3, & 4

NTX, Naltrexone; DID, Modified drinking in the dark; CON, Control; SA, Self-administration; SC, Subcutaneous injection; PR, Progressive ratio.