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Differential effects of ghrelin antagonists on alcohol drinking and reinforcement in mouse and rat models of alcohol dependence

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ABSTRACT

An effort has been mounted to understand the mechanisms of alcohol dependence in a way that may allow for greater efficacy in treatment. It has long been suggested that drugs of abuse seize fundamental reward pathways and disrupt homeostasis to produce compulsive drug seeking behaviors. Ghrelin, an endogenous hormone that affects hunger state and release of growth hormone, has been shown to increase alcohol intake following administration, while antagonists decrease intake. Using rodent models of dependence, the current study examined the effects of two ghrelin receptor antagonists, [DLys3]-GHRP-6 (DLys) and JMV2959, on dependence-induced alcohol self-administration. In two experiments adult male C57BL/6J mice and Wistar rats were made dependent via intermittent ethanol vapor exposure. In another experiment, adult male C57BL/6J mice were made dependent using the intragastric alcohol consumption (IGAC) procedure. Ghrelin receptor antagonists were given prior to voluntary ethanol drinking. Ghrelin antagonists reduced ethanol intake, preference, and operant self-administration of ethanol and sucrose across these models, but did not decrease food consumption in mice. In experiments 1 and 2, voluntary drinking was reduced by ghrelin receptor antagonists, however this reduction did not persist across days. Despite the transient effects of ghrelin antagonists, the drugs had renewed effectiveness following a break in administration as seen in experiment 1. The results show the ghrelin system as a potential target for studies of alcohol abuse. Further research is needed to determine the central mechanisms of these drugs and their influence on addiction in order to design effective pharmacotherapies.

1. Introduction

Alcohol dependence is a disorder that affects millions of people worldwide. Current treatments for dependence include both behavioral approaches and pharmacological agents such as naltrexone and acamprosate. However, most treatments have drawbacks and issues with compliance. Thus, novel treatments are being developed and researched with the intention of improving effectiveness. Interests have turned to the gut hormone ghrelin, a gastric peptide that plays a key role in hunger and growth regulation through actions on the growth hormone secretagogue receptors [GHSR] (Kojima et al., 1999). Attention has been focused on how the ghrelin system affects use and abuse of addictive substances.

The ghrelin system appears to be highly associated with alcohol dependence in humans. Humans diagnosed with alcohol dependence show decreased plasma and fundic ghrelin levels at the onset of alcohol withdrawal when compared to matched healthy controls (Badaoui et al., 2008). In contrast, alcoholics show elevated circulating ghrelin levels after 30 days of abstinence (Kim et al., 2013),

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suggesting a compensatory increase in ghrelin production during recovery from withdrawal. Furthermore, there is a positive correlation between ghrelin levels and alcohol craving (Addolorato et al., 2006; Leggio et al., 2012). Congruently, intravenous ghrelin increases alcohol craving scores in alcoholics (Leggio et al., 2014). Oral consumption of alcohol exerts an acute inhibitory effect on ghrelin secretion in non-dependent men and women (Calissendorff et al., 2005, 2012). Despite ample evidence of interaction between the ghrelin system and alcohol consumption, testing of ghrelin receptor antagonists is still in early stages of development.

In rodent models, central administration of ghrelin increases alcohol consumption (Jerlhag et al., 2009) and peripheral administration induces conditioned place preference (CPP) and increases dopamine levels the nucleus accumbens (Jerlhag, 2008), suggesting that ghrelin influences alcohol consumption via reward-related circuitry. Compounds that block the ghrelin receptor GHSR1a generally show effects opposite to those of ghrelin agonists on alcohol drinking and reward. For example, the small molecule ghrelin receptor antagonist JMV2959 decreased ethanol intake (Jerlhag et al., 2009) and blocked ethanol-induced CPP (Bahi et al., 2013). Similarly, Kaur and Ryabinin (2010) found that the peptide-based ghrelin antagonist, [DLys3]-GHRP-6 (DLys), decreased ethanol intake and preference, while also blocking ethanol induced c-Fos immunoreactivity in the Edinger–Westphal nucleus. Models using ghrelin knockout mice have seen a reduction in alcohol-induced reactions, which include decreased locomotor effects and reduced accumbal dopamine release following alcohol administration (Jerlhag and Engel, 2011).

Studies that utilize ghrelin receptor antagonists to assess effects on alcohol drinking have generally tested acute effects in non-dependent animals. Dependence models of alcohol consumption may be better suited for understanding the role ghrelin plays in the processes that contribute to alcoholism in humans. Recently, Suchankova et al. (2013) addressed the topic of alcohol dependence by examining the alcohol deprivation effect with and without administration of JMV2959. They found that JMV2959 blocks the increased ethanol intake seen following reintroduction of ethanol. However, the effect of ghrelin antagonism in models of alcohol dependence needs further evaluation.

A number of alcohol dependence models using mice and rats have been developed (review; Knapp and Breese, 2012). Among them are studies using alcohol vapor or intragastric exposure. Continuous and intermittent ethanol vapor exposure has been shown to reliably produce signs of dependence (e.g., Schulteis et al., 1995, 1996; Macey et al., 1996; Becker, 2000), to increase alcohol drinking and operant self-administration of alcohol (e.g., Becker and Lopez, 2004; O'Dell et al., 2004; Finn et al., 2007; Gilpin et al., 2009), and to increase alcohol self-administration to alleviate withdrawal symptoms (Roberts et al., 1996) and during a period of protracted abstinence (Roberts et al., 2000). More recently, the intragastric alcohol consumption (IGAC) model has shown increased alcohol self-administration in dependent mice given ethanol access during acute alcohol withdrawal (Fidler et al., 2012; Cunningham et al., 2013).

The current study tested the ability of two ghrelin receptor (GHSR1a) antagonists, DLys and JMV2959, to decrease alcohol drinking across three procedures: 1) alcohol drinking after chronic intermittent ethanol vapor exposure in C57BL/6J mice (Finn et al., 2007); 2) intragastric (IG) alcohol self-infusion after passive IG alcohol infusions in C57BL/6J mice (Fidler et al., 2012); and 3) progressive ratio (PR) operant alcohol self-administration in Wistar rats after chronic intermittent ethanol vapor (O'Dell et al., 2004). These subjects and models were chosen to provide a wide array of examinations to test the effectiveness of ghrelin antagonists and their interaction with alcohol drinking. The models used were developed at different times in different laboratories, and therefore, differ in many procedural parameters. Our main goal was to test the consistency of GHSR1a antagonists across these different models without attempting to match all the parameters. Mice were used in experiment 1 and 2 as a way of testing two different models of dependence, while rats were used in experiment 3 as a way of testing a different species with a similar dependence model allowing for comparison to experiment 1. We believe that using both rats and mice would allow us to determine the effectiveness of ghrelin receptor antagonists across species, thereby enhancing the generalizability of our findings. The general hypotheses for these experiments was that both of the ghrelin antagonists would reduce the expected alcohol self-administration levels in dependent animals.

2. Methods

2.1. Animals

Male C57BL/6J mice (N = 96) from Jackson Laboratories (Sacramento, CA) and male Wistar rats (N = 12) from Charles River (Wilmington, MA) were used in these experiments. All mice and rats had *ad libitum* access to food and water, except for 16–23 h before IG cannulation in experiment 2. The Oregon Health & Science University Institutional Animal Care and Use Committee (IACUC) approved all procedures for experiments 1 and 2. Baylor College of Medicine IACUC approved all procedures for experiment 3.

In experiment 1, one week after arrival (6–7 weeks old), acclimation to the environment, and switch to a 12 h reverse light cycle (*off at* 08:00), mice were randomly assigned to one of six groups (n = 8/group) in a 2 × 3 design. Mice were individually housed and a baseline of limited access (2 h) alcohol intake was established. Then separate groups of mice were exposed to either ethanol vapor or air vapor for one cycle (16 h on/8 h off × 3 days), followed by 2 h alcohol intake for 5 days, a second cycle of intermittent ethanol vapor or air exposure, and a final 5 days of 2 h alcohol intake. Prior to each of the post-vapor alcohol intake days, subgroups of mice were pre-treated with saline, DLys (15 mg/kg), or JMV2959 (9 mg/kg).

In experiment 2, mice arrived at 8–9 weeks of age and were allowed to acclimate for at least 1 week before surgery. Mice were initially group housed (4/cage) on a 12 h light/dark cycle (*off* at 19:00). After surgery and recovery, mice were singly housed in operant conditioning chambers for the rest of the experiment. Mice initially received passive-infusions of either ethanol or water over 10 days (n = 12/group). Ethanol self-infusion was then measured daily for 23.5 h after IG administration of either DLys (14–18.2 mg/kg) or saline.

In experiment 3, rats were split into two groups (n = 6/group) and trained to lever press for solutions of ethanol or sucrose on a fixed ratio schedule. Rats weighed over 400 g at the start of the experiment and were housed individually on a 12 h light cycle (*off* at 18:00). Following intermittent ethanol vapor exposure, rats were then administered DLys (0, 2, 4 mg/kg) or JMV2959 (0, 1, 2, 4 mg/kg) and operant self-administration of ethanol or sucrose was tested under a PR schedule in a within-subjects design.

2.2. Drugs

Two ghrelin receptor (GHSR1a) antagonists were used for these experiments: [DLys3]-GHRP-6 (Tocris Bioscience, Bristol, UK; Cat #1922) and JMV2959 (Aeterna Zentaris, Germany). In experiments 1 and 3, drugs were diluted in sterile saline (0.9% sodium chloride). In experiment 2, sterile water was used as the vehicle. All solutions were prepared fresh daily and different doses of drugs were used in mice and rats based on previously published experiments (Jerlhag et al., 2009; Kaur and Ryabinin, 2010; Gomez and Ryabinin, 2014).

In experiment 1, DLys and JMV2959 were injected intraperitoneally (i.p.) at 15 mg/kg and 9 mg/kg, respectively. Ethanol (95% pure) was diluted with tap water to 15% v/v and presented using 25 ml graduated cylinders capped with a sipper tube. An alcohol dehydrogenase inhibitor, pyrazole HCl (68.1 mg/kg) with a priming dose of ethanol (2 g/kg, 20% v/v ethanol solution) was used prior to vapor exposure of mice to allow for exposure to lower concentrations of vaporized alcohol and gain consistent blood ethanol concentrations (BECs) across subgroups.

In experiment 2, DLys was administered IG at a set dose of 400 nmol/0.3 ml per mouse (which translates to 14–18.2 mg/kg given the range of body weights) or vehicle (0.3 ml) 30 min before each daily session during the No-Choice and Choice phases when 20% ethanol (v/v in sterile water) was self-infused.

In experiment 3, rats were administered three doses of DLys (0, 2, 4 mg/kg) and four doses of JMV2959 (0, 1, 2, 4 mg/kg). Rats were given 0.1 ml of 10% ethanol or 2% sucrose per delivery during operant conditioning sessions.

2.3. Apparatus

In experiment 1, mice were housed in flow-through cages (3–4 per cage) in each vapor inhalation chamber (Flair Plastics, Portland, OR). Immediately before each