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Brief communication

Ghrelin receptor (GHS-R1A) antagonism alters preference for ethanol and sucrose in a concentration-dependent manner in prairie voles

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ABSTRACT

Ghrelin receptor (GHS-R1A) activity has been implicated in reward for preferred foods and drugs; however, a recent study in our laboratory indicated that GHS-R1A antagonism reduces early (after only four exposures) preference for 20% ethanol, but not 10% sucrose in prairie voles, a genetically diverse high alcohol-consuming species. The purpose of the present study was to determine if these effects of GHS-R1A antagonism depend on the concentration of the rewarding solution being consumed. We first characterized preference for varying concentrations of ethanol and sucrose. Two bottle tests of each ethanol concentration versus water indicated that 10% and 20% ethanol are less preferred than 3% ethanol, and a follow-up direct comparison of 10% vs. 20% showed that 10% was preferred over 20%. Direct two-bottle comparisons of 2% vs. 5%, 2% vs. 10%, and 5% vs. 10% sucrose showed that 10% sucrose was most preferred, and 2% sucrose was least preferred. The effects of JMV 2959, a GHS-R1A antagonist, on preference for each concentration of ethanol and sucrose were then tested. In a between groups design prairie voles were given four two-hour drinking sessions in which animals had access to ethanol (3, 10, or 20%) versus water, or sucrose (2, 5, or 10%) versus water every other day. Saline habituation injections were given 30 min before the third drinking session. JMV 2959 (i.p.; 9 mg/kg), a GHS-R1A antagonist, or saline was administered 30 min before the fourth drinking session. JMV 2959 reduced preference for 20% ethanol and 2% sucrose, but had no significant effect on preference for the other ethanol and sucrose concentrations. These data identify constraints on the role of GHS-R1A in early preference for ethanol and sucrose, and the concentration-dependent effects suggest strong preference for a reward may limit the importance of GHS-R1A activity.

1. Introduction

Ghrelin is an orexigenic neuropeptide that has been shown to play a key role in reward and hedonic eating [1–8]. Ghrelin can act via the growth hormone secretagogue receptor 1A (GHS-R1A) to enhance dopamine release in the nucleus accumbens, particularly in response to reward-predictive cues [9–11], and dopamine release in the nucleus accumbens is thought to be a central component of reward signaling in the brain [12].

The reward-mediating effects of ghrelin are further demonstrated by studies showing that ghrelin administration can increase intake of and motivation for drug and non-drug rewards in rodents. Ghrelin administration can increase the consumption of sweets [13] and increase conditioned place preference for a high fat diet [14]. The mesolimbic dopamine system has been shown to be the key cite of action for the effects of ghrelin on reward-based consumption of food and food motivation [15–19], and the effects of ghrelin on the consumption of rewarding foods has been shown to be independent of the caloric content of food [20]. Ghrelin administration into either the ventricles or the VTA also increases the consumption of ethanol, a drug reward [21]. Conversely, pharmacological blockade of GHS-R1A attenuates reward-induced dopamine release in the nucleus accumbens [22–24]. Further, GHS-R1A antagonism reduces consumption of rewarding foods [13,15–18,25]. GHS-R1A administration also reduces ethanol consumption and decreases ethanol-induced dopamine release in the nucleus accumbens [26–30]. In order to investigate GHS-R1A as a potential target for the treatment of addiction and obesity, most of these studies of GHS-R1A antagonism have followed long-term consumption of rewards to better model the effects of extended or pathological consumption of rewarding substances.

In contrast to those long-term exposure studies, we recently investigated the role of ghrelin in early reward for ethanol and sucrose, with

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the goal of characterizing ghrelin's contribution to basic or innate (non-pathological) reward mechanisms. Here, early is defined as following only brief exposure to ethanol (four two-hour sessions). Additionally, understanding the mechanisms of early preference for rewarding substances like sugar and ethanol is important because initial responses could predict or contribute to overconsumption. We showed that GHS-R1A antagonism reduced early preference for 20% ethanol but not 10% sucrose in prairie voles [31], a genetically diverse animal model of high alcohol consumption [32]. These data suggested limitations on ghrelin's role in reward. We hypothesized that ghrelin plays a smaller role in the early consumption of substances with high hedonic value, such as 10% sucrose. Therefore, the goal of this study was to determine if the effects of GHS-R1A antagonism depend on the concentration of the rewarding solution being consumed.

To determine if the concentration of rewarding substances influences the impact of GHS-R1A antagonism on preference, the GHS-R1A antagonist JMV 2959 or vehicle was administered prior to the fourth two-bottle drinking session (20%, 10%, or 3% ethanol versus water, or 10%, 5%, or 2% sucrose versus water). Preference for 20% ethanol and 2% sucrose were reduced by GHS-R1A antagonism, but preference for all other ethanol and sucrose concentrations was unaffected. These data show that the effects of GSH-R1A antagonism vary with the concentration of ethanol or sucrose. As 20% ethanol and 2% sucrose were determined to be the least preferred concentrations of each substance, these data may indicate that GHS-R1A activity has a larger role in the reward for substances with relatively lower hedonic value.

2. Materials & methods

2.1. Subjects

188 pair-housed female prairie voles (*Microtus ochrogaster*) were used for the study (n = 10 for each treatment group). Females were used in order to follow up on our previous study of JMV 2959 effects in female prairie voles [31]. Animals had access to water and custom rabbit chow ad libitum. The animals were naïve to sucrose and ethanol prior to testing. Different animals were used for each solution concentration condition. In order to measure individual consumption, animals were moved into individual cages 30 min prior to each drinking session, then returned to their home cages with their siblings immediately following the session. The home and experimentation rooms were maintained at 20–23 °C on a 14/10-hour light/dark cycle (lights on at 6:00). All procedures were approved by the Institutional Animal Care and Use Committee (IACUC) at Bucknell University and complied with the Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research (National Research Council 2003).

2.2. Drugs

GHS-R1A antagonist JMV 2959 (EMD Millipore, Billerica, MA) dissolved in 0.9% saline was injected intraperitoneally (0.0 or 9.0 mg/kg; 0.9 mg/mL). We have previously shown that this dose is effective at reducing ethanol preference in prairie voles [31]. Injection volumes for both JMV 2959 and saline were 10 mL/kg.

2.3. Procedure

2.3.1. Experiment 1: characterization of preference for 3%, 10%, or 20% ethanol vs. water

In order to determine the relative preference for each concentration of ethanol in prairie voles, 8 animals (ages 2–10 months) experienced 2-h two-bottle drinking sessions once a day for a total of 8 sessions. Animals were taken from their pair-housed home cages at 16:00, and placed into individual drinking cages with standard chow. Two modified graduated cylinders filled with an ethanol concentration and water, respectively, were inserted into each cage and initial measurements of their volumes were recorded immediately. At the conclusion of each session, final volumes were read, and animals were returned to their home cages. Every animal was tested on each of the three ethanol solutions twice in descending then ascending concentration order, once on the left and once on the right position to avoid side preferences. To further investigate if there was a difference in preference ratio for 10% versus 20%, animals were given 24-hour access to one bottle of each bottle of 10%, one bottle of 20%, as well as their regular drinking water. Preference ratio was calculated based on the final volume readings [mL ethanol consumed / (mL ethanol + mL water consumed)]. Consumption of each ethanol solution for each cage was averaged across three days. Note that consumption here reflects the amount of solution consumed by both animals in the cage. 24-hour testing allowed for a more clear indication of which solution was preferred, and we did not want to isolate the animals for such an extended period of time. However, cage-mates were size-matched littermates. Furthermore, because voles show social facilitation of drinking, and tend to consume similar amounts to their cage-mates [32]. Unpublished observations in our lab also indicate a high correlation for ethanol consumption between cage-mates. Therefore, we believe these preference ratios likely reflect the preference of each animal in the cage.

2.3.2. Experiment 2: effects of JMV 2959 on limited-access consumption of 3%, 10%, or 20% ethanol vs. water

Animals (ages 4–11 months) were pseudo-randomly assigned to three separate groups so that ages were evenly distributed across groups. Animals experienced 2-h two-bottle drinking sessions once a day for a total of 4 sessions. Each group was assigned one of the three ethanol concentrations (3%, 10%, or 20%; n = 18 for each concentration) for all four of the sessions.

Animals were taken from their pair-housed home cages at 15:30, and placed into individual drinking cages with standard chow. At 16:00, 2 modified graduated cylinders filled with water and the designated ethanol solution (3%, 10%, or 20%) were inserted into each cage, and initial measurements of their volumes were recorded immediately. The position of the ethanol and water bottles was alternated each session to avoid side preferences. Volumes were read at 1 and 2 h. At the conclusion of the session, animals were returned to their home cages with their partners. Saline habituation injections were administered 30 min before the third session, and JMV 2959 pre-treatment (0.0 or 9.0 mg/kg) occurred 30 min before the final (fourth) session. Animals were sorted into JMV 2959 treatment groups based on their ethanol consumption and preference ratio from session 2, such that treatment groups were matched for ethanol consumption and preference ratio. Additionally, ages were evenly distributed across treatment groups. Preference ratio was calculated based on the final volume readings [mL ethanol consumed / (mL ethanol + mL water consumed)].

2.3.3. Experiment 3: characterization of preference for 2%, 5%, and 10% sucrose using two-bottle direct comparisons

8 animals (ages 4–8 months, previously used in Experiment 2) experienced 2-h two-bottle drinking sessions once a day for a total of 6 sessions. Because all sucrose concentration yielded nearly 100% preference ratios when compared to water, direct comparisons between sucrose concentrations provided a more meaningful description of the concentration/preference relationship. Therefore, to characterize preference for each concentration of sucrose, animals were taken from their pair-housed home cages at 16:00, and placed into individual drinking cages with standard chow. 2 modified graduated cylinders filled with different concentrations of sucrose (2%, 5%, or 10%) were inserted into each cage and initial measurements of their volumes were recorded immediately. Sucrose comparisons were presented in the following order: 2% versus 5%, 2% versus 10%, and 5% versus 10%, then each comparison was repeated once more in the reverse order with the opposite left–right bottle position of each concentration. At the conclusion of each session, final volumes were read, and animals