Intravenous Ghrelin Administration Increases Alcohol Craving in Alcohol-Dependent Heavy Drinkers: A Preliminary Investigation

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Background: There is a need to identify novel pharmacologic targets to treat alcoholism. Animal and human studies suggest a role for ghrelin in the neurobiology of alcohol dependence and craving. Here, we were the first to test the hypothesis that intravenous administration of exogenous ghrelin acutely increases alcohol craving.

Methods: This was a double-blind, placebo-controlled human laboratory proof-of-concept study. Nontreatment-seeking, alcohol-dependent, heavy-drinking individuals were randomized to receive intravenous ghrelin 1 mcg/kg, 3 mcg/kg or 0 mcg/kg (placebo), followed by a cue-reactivity procedure, during which participants were exposed to neutral (juice) and alcohol cues. The primary outcome variable was the increase in alcohol craving (also called urge) for alcohol, assessed by the Alcohol Visual Analogue Scale.

Results: Out of 103 screenings, 45 individuals received the study drug. Repeated measures of analysis of covariance revealed a group effect across ghrelin doses in increasing alcohol craving ($p < .05$). A dose-specific examination revealed a significant effect of ghrelin 3 mcg/kg versus placebo in increasing alcohol craving ($p < .05$) with a large effect size ($d = .94$). By contrast, no significant ghrelin effect was found in increasing either urge to drink juice or food craving ($p =$ ns). No significant differences in side effects were found ($p =$ ns).

Conclusions: Intravenous administration of exogenous ghrelin increased alcohol craving in alcohol-dependent heavy-drinking individuals. Although the small sample requires confirmatory studies, these findings provide preliminary evidence that ghrelin may play a role in the neurobiology of alcohol craving, thus demonstrating a novel pharmacologic target for treatment.

Key Words: Alcoholism, craving, cue-reactivity, feeding peptides, ghrelin, neuroendocrinology

Alcoholism is one of the leading causes of mortality and morbidity (1,2). Therefore, interventions for alcoholism may have important implications. Hence, there is a need to identify new pathways that may serve as pharmacologic targets for treatment (3).

Ghrelin is a 28-amino-acid peptide acting as the endogenous ligand for the growth hormone secretagogue receptor (GHS-R1a) (4). Ghrelin activates hypothalamic orexigenic neurons and inhibits anorectic neurons to induce hunger and stimulate feeding (5,6). Growth hormone secretagogue receptors are highly co-expressed with dopamine receptors in the midbrain, raphe nuclei, and ventral tegmental area (7–9), suggesting that ghrelin modulates reward processing. In mice, ghrelin administration intraperitoneally (10) or centrally into the ventral tegmental area (11,12) activates measures associated with reward.

Preliminary human studies show differences in endogenous blood ghrelin levels in actively drinking (13–15) and abstinent alcoholics (16,17) and changes in ghrelin levels over time based on the drinking status (18). Some studies indicate a significant positive correlation between ghrelin levels and alcohol craving [(13,18,19) but see (17)]. However, while animal studies tested the direct effects of exogenous ghrelin administration, human studies were limited to measuring endogenous blood ghrelin levels and retrospective measures of alcohol craving, thus significantly limiting their bench-to-bedside value (20). There is no human evidence that manipulations of ghrelin signaling via administration of exogenous ghrelin increases alcohol-seeking behaviors, such as alcohol craving. This was the first study testing this hypothesis.

Methods and Materials

Subjects

Design and Setting. This was a three-group between-subject, double-blind, placebo-controlled randomized human laboratory proof-of-concept study, conducted at the Brown University Center for Alcohol and Addiction Studies, Providence, Rhode Island. The study was approved by the Brown University Institutional Review Board. The use of synthetic human ghrelin was approved under the Food and Drug Administration Investigational New Drug 109,242.

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Study Drug. Current Good Manufacturing Practice human acetylated ghrelin was purchased from PolyPeptide Laboratories (Torrance, California). The purity of the peptide was >95% and its authenticity was confirmed by mass spectrometry, tandem mass spectrometry analysis, and amino acid analysis. The final ghrelin solution demonstrated excellent stability after 72 hours and was sterile and free of detectable pyrogens. The final solution was always prepared <48 hours before its administration.

Study Population. Nontreatment-seeking alcohol-dependent men and women were screened according to inclusion/exclusion criteria (Supplement 1).

Study Overview. Potential participants recruited via mass media were prescreened by phone. Potentially eligible participants came to our facility. After complete description of the study, written informed consent was obtained and then screening took place (visit 1). Eligible participants were randomized to ghrelin 1 mcg/kg, 3 mcg/kg, or 0 mcg/kg (placebo) and the experimental session (visit 2) was scheduled. The experimental session consisted of a ~10-minute administration of ghrelin/placebo, followed by a cue-reactivity procedure. A breath alcohol concentration of .00 was required at each visit. Compensation in the form of cash was provided at each visit.

Experimental Session. The session was conducted individually in a one-way mirror room. Subjects came to our laboratory after having fasted. An intravenous cannula was placed, and a fixed light breakfast was served, i.e. ~700 kJ, approximately 62% carbohydrate, 13% protein, and 25% fat (11,21–23). Consistent with previous cue-reactivity studies (24,25), participants were exposed to visual, tactile, olfactory, and proprioceptive stimuli associated with neutral and alcohol beverages. As for the neutral condition, a palatable nonalcoholic beverage, rather than water (24,25), was included. Specifically, consistent with Rohsenow *et al.* (26), a juice condition was used, i.e., a 295 mL bottle of a commercially available fruit punch. Additionally, food craving was assessed via the General Food Cravings Questionnaire-State (27).

Before intravenous ghrelin/placebo administration, participants underwent a 3-minute relaxation period to collect baseline levels of urge and physiological arousal (Table 1). Then, a staff member entered the room with a tray, covered by an inverted pitcher, containing the fruit punch bottle and a glass. The pitcher was removed, the bottle was opened, and the glass was filled. The staff member left the room, and an audiotape instructed the participant to sniff the glass of juice when they heard high tones and stop sniffing when they heard low tones. This procedure included thirteen 5-second olfactory exposures during each 3-minute trial. Next, participants underwent a 3-minute alcohol cue exposure that was identical to the previous procedure except the juice bottle was replaced with the appropriate commercially labeled alcohol bottle. After the stimuli were removed, a relaxation period took place, and a second juice trial and finally a second alcohol trial were presented (Table 1). For urge, Alcohol Visual Analogue Scale and Juice Visual Analogue Scale were rated on 11-point anchored Likert-type scales (26). The Alcohol Attention Scale was used to assess attention to sight/smell of alcohol cues (24). We also assessed attention to sight/smell of juice cues, adapting the Alcohol Attention Scale (i.e., Juice Attention Scale).

Previous studies indicate that alcohol cue-elicited craving may be associated with parallel increases in mean arterial pressure (MAP), heart rate (HR), and salivation (28). In this study, we measured HR and salivation as secondary outcomes, while we monitored MAP only for safety reasons. In fact, a reduction of blood pressure is a possible common transitory side effect of intravenous ghrelin; therefore, blood pressure might have exhibited low validity in our paradigm. Mean arterial pressure and HR were obtained using using a monitoring machine. As for measuring salivation, participants placed three dental rolls in their mouths; rolls were weighed immediately before and after the cue-reactivity with an analytical scale, so that the net difference indicated the saliva mass produced during cue-reactivity (29). After all procedures were completed, a meal was provided, a postsession debriefing was performed, and then participants were released. The Systematic Assessment for Treatment Emergent Events was used for adverse events (30,31). Approximately a week after visit 2, a brief safety follow-up (visit 3) took place, during which a brief motivational session to reduce alcohol use was provided, based on the motivational enhancement therapy manual (32).

Blood Samples Analysis. Blood samples were collected at six time points (Table 1), centrifuged and stored at −80°C. Samples were analyzed together at the end of the study to maintain the blind. Total serum ghrelin levels were determined using a fluorescent bead-based Bio-Plex assay (Bio-Rad, Hercules, California) following the manufacturer's protocol. Results were expressed as pg/mL.

Statistical Analysis

Preliminary analyses included the examination of the distributions of the outcome measures. All outcome measures

Table 1. Study Activities and Timeline During the Experimental Session

Time Point (Minutes)	Procedures and/or Assessments
−40	Breakfast (~700 kJ)
−15	Baseline: Blood Sample #1, Urge to Drink Alcohol, Urge to Drink Juice, Food Craving
−13	Baseline: Mean Arterial Pressure, Heart Rate
−10	Study Drug Intravenous (IV) Administration: Ghrelin 1 mcg/kg, Ghrelin 3 mcg/kg, or 0 mcg/kg (Placebo)
+3	Juice Trial #1: Mean Arterial Pressure, Heart Rate, Saliva Mass
+6	Post-Juice Trial #1: Urge to Drink Juice, Juice Attention Scale, Blood Sample #2, Relaxation Period
+9	Alcohol Trial #1: Mean Arterial Pressure, Heart Rate, Saliva Mass
+17	Post-Alcohol Trial #1: Urge to Drink Alcohol, Alcohol Attention Scale, Blood Sample #3, Adverse Events, Food Craving, Relaxation Period
+20	Juice Trial #2: Mean Arterial Pressure, Heart Rate, Saliva Mass
+23	Post-Juice Trial #2: Urge to Drink Juice, Juice Attention Scale, Blood Sample #4, Relaxation Period
+26	Alcohol Trial #2: Mean Arterial Pressure, Heart Rate, Saliva Mass
+29	Post-Alcohol Trial #2: Urge to Drink Alcohol, Alcohol Attention Scale, Blood Sample #5, Adverse Events
+46	Relaxation Period
+48	Postexperiment Assessment: Blood Sample #6, Urge to Drink Alcohol, Food Craving