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Pharmacologic Antagonism of Ghrelin Receptors Attenuates Development of Nicotine Induced Locomotor Sensitization in Rats

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Abstract

Aims—Ghrelin (GHR) is an orexigenic gut peptide that interacts with ghrelin receptors (GHR-Rs) to modulate brain reinforcement circuits. Systemic GHR infusions augment cocaine

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Authors contribution

PSC and PJW were responsible for the study concept and design. JR, SH, and PSC collected the locomotor data. PSC and PJW assisted with data analysis and interpretation of findings. PJW drafted the manuscript. PSC, JR, SE, and J-AF provided critical revision of the manuscript. All authors critically reviewed content and approved the final version for publication.

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stimulated locomotion and conditioned place preference (CPP) in rats, whereas genetic or pharmacological ablation of GHR-Rs has been shown to attenuate the acute locomotor-enhancing effects of nicotine, cocaine, amphetamine and alcohol and to blunt the CPP induced by food, alcohol, amphetamine and cocaine in mice. The stimulant nicotine can induce CPP and like amphetamine and cocaine, repeated administration of nicotine induces locomotor sensitization in rats. A key issue is whether pharmacological antagonism of GHR-Rs would similarly attenuate nicotine-induced locomotor sensitization.

Method—To examine the role of GHR-Rs in the behavioral sensitizing effects of nicotine, adult male rats were injected with either 0, 3 or 6 mg/kg of the GHR-R receptor antagonist JMV 2959 (i.p.) and 20 minutes later with either vehicle or 0.4 mg/kg nicotine hydrogen tartrate (s.c.) on each of 7 consecutive days.

Results—Rats treated with nicotine alone showed robust locomotor sensitization, whereas rats pretreated with JMV 2959 showed significantly attenuated nicotine-induced hyperlocomotion.

Conclusions—These results suggest that GHR-R activity is required for the induction of locomotor sensitization to nicotine and complement an emerging literature implicating central GHR systems in drug reward/reinforcement.

Key terms

ghrelin; ghrelin receptors; JMV 2959; locomotion; sensitization

1. Introduction

The 28 amino acid hormone ghrelin (GHR) is derived from a prehormone secreted from endocrine cells of the stomach and gut. GHR is unique in that this hormone undergoes a posttranslational modification in which an octanoate group is added to the third serine group to form acylated-GHR. Acyl-GHR (hereafter referred to as GHR) is the endogenous ligand for the growth hormone secretagogue 1a receptor (GHS-R1a or GHR-R) [1–2]. GHR-Rs are located in the periphery and CNS. The peripheral actions of ghrelin include alteration of gastric emptying [3–4], activation of vagal afferents [3] and reduced inflammatory reactions [4]. Systemic GHR is passively transported across the blood–brain barrier [5–7]. GHR-Rs are located on neurons within the arcuate nucleus, the hippocampus and the ventral tegmental area (VTA) [6, 8–12]. These CNS sites are importantly linked to the control of eating, induction of growth hormone release, modulation of memory and to reinforcement, respectively [2, 6, 8, 13–15].

An emerging literature strongly suggests that GHR and GHR-Rs are positioned so as to modulate reinforcement to addictive drugs and natural reinforcers that act via modulation of brain dopamine circuits [8, 15–17]. Systemic or central administration of GHR enhances food intake [18–20] and augments feeding-associated behaviors such as hoarding and foraging [21]. Acute changes in peripheral GHR levels can also induce changes in brain dopamine signaling. Abizaid and colleagues noted that systemic administration of GHR can reorganize VTA neurons such that these cells express more excitatory input and diminished inhibitory input [9]. Systemic GHR administration induces excitation of VTA neurons [9] and systemic GHR injection increases dopamine overflow within the nucleus accumbens [9, 22], particularly within the shell region, but not the core region, of the nucleus accumbens [23]. Consistent with these effects are reports in which systemic administration of GHR enhanced cocaine-induced hyperlocomotion [24] and chronic daily injection of GHR in rats induced a degree of locomotor sensitization to a subsequent injection of cocaine [25]. More importantly, systemic and central administration of GHR can induce conditioned place preference (CPP) *per se* [22, 26], as well as enhance CPP induced by cocaine and by food

[16, 27–28]. Tessari and colleagues [29] reported that circulating GHR levels were positively related to the reinstatement of responding for intravenous cocaine. These studies indicate that augmenting GHR levels can facilitate the behavioral actions of cocaine and in particular may play a role in the sensitizing effects of drugs of abuse.

An alternative strategy to the aforementioned involves the assessment of addictive drug effects in animals sustaining inactivation of either GHR or GHR-Rs. Pharmacological antagonists of GHR-Rs have been developed, in part, because of the linkage of GHR-R activation to induction of feeding, with the hope that GHR-R inactivation might represent a therapeutic approach for the treatment of obesity. One such drug is JMV 2959, a selective competitive GHR-R antagonist [30–32]. JMV 2959 binds to GHR-Rs with low nanomolar affinity [31]. As would be expected of a GHR-R antagonist, s.c. administration of JMV 2959 dose-dependently blocked the feeding response induced by the synthetic GHR agonist hexarelin [30]. JMV 2959 represents an important tool for the role of GHR-Rs in drug abuse. Importantly, pharmacological inactivation of GHR-R's by JMV 2959 has been noted to attenuate or to ablate the acute locomotor and CPP properties of amphetamine, cocaine, ethanol, and most recently that of nicotine [26, 33–35]. A specific recent focus of our laboratory has been on the impact of pharmacological blockade of GHR-Rs on the development of locomotor sensitization induced by repeated drug exposure. In the present study, we considered the impact of JMV 2959 on the development of locomotor sensitization induced by daily administration of nicotine in rats.

2. Materials and Methods

2.1 Animals

Animal research was conducted in accordance with the guidelines provided by the Texas A&M University Laboratory Animal Care Committee. The subjects of this experiment were 40 adult male Sprague-Dawley rats obtained from Harlan (Houston, Texas, USA) weighing 250–275 g at the start of the experiment. Our previous studies of cocaine sensitization employed this strain of rats [36–37]. The rats were acclimated to the colony for a week prior to the start of the study. All animals were housed individually in polycarbonate cages with continuous access to food and tap water (except during locomotion testing). The colony room temperature was maintained at $21 \pm 1^\circ\text{C}$ while the humidity was maintained at 60–70%. The colony room was maintained on a 12-hour light/dark cycle (lights on at 0900 hrs) and behavioral testing commenced at approximately 0900 hrs.

2.2 Drugs

Nicotine was prepared by dissolving nicotine hydrogen tartrate salt (HT: Sigma, St. Louis, MO, USA) into 0.9% NaCl at a concentration of 0.4 mg/1.0 ml (dose calculated as the free base). The pH of the nicotine solution was adjusted to ~ 7.0 using sodium hydroxide. The nicotine dose was chosen on the basis of results from prior studies noting the induction of robust locomotor sensitization in rats at this dose [38–41]. JMV 2959 HCl was dissolved into 0.9% NaCl at a concentration of 3.0 and 6.0 mg/ml (dose calculated as the salt) and was administered in a volume of 1.0 ml/kg. The JMV 2959 doses were chosen on the basis of preliminary work in this lab which documented attenuation of cocaine-induced sensitization (Clifford et al., 2011, in press) and studies by Jerlhag and colleagues documenting significant attenuation by JMV 2959 pretreatment of acute hyperlocomotion elicited in mice by cocaine, amphetamine or nicotine [26, 34].

2.3 Apparatus

The assessment of locomotion was made in a set of 8 automated optical beam activity monitors (Model RXYZCM-16; Accuscan Instruments, Columbus, OH, USA). Each

monitor is housed within a 40 × 40 × 30.5 cm acrylic cage. Activity monitors and cages were located in a sound-proof room with a 40 dB [SPL] white noise generator operating continuously. A multiplexor-analyzer monitored beam breaks from the optical beam activity monitors and tracked the simultaneous interruption of beams. The multiplexor-analyzer updated the animal's position in the acrylic cage every 10 msec using a 100% real-time conversion system. Computerized integration of the data obtained from the monitor afforded the recording of general activity using total distance (in cm) as the primary dependent measure [42].

2.4 Procedures

On two consecutive days, the rats were adapted to the locomotion chambers for 60 minutes per day. On the next three days, the rats were injected (i.p.) with 0.9% saline (1 ml/kg) 5 minutes before being placed into the activity chamber. The last day of these vehicle injection trials served as day 0 for baseline. The rats were placed into the locomotion chambers for 15 minutes, removed and then injected with 0.9% saline and then placed back into the chambers for an additional 45 minutes. During the 7 day nicotine exposure period, a third of the rats in each nicotine injection condition were treated (i.p.) with either vehicle (0), 3 or 6 mg/kg JMV 2959 at 5 minutes before being placed into the locomotion chambers. After the 15 minute baseline period, the rats were injected (s.c.) with either saline or 0.4 mg/kg nicotine HT and placed back into the locomotion chamber for 45 minutes. This pretreatment-treatment combination formed six test groups: vehicle-vehicle (n=6), vehicle-nicotine (n=6), 3 mg/kg JMV 2959-vehicle (n=8), 3 mg/kg JMV 2959-nicotine (n=8), 6 mg/kg JMV 2959-vehicle (n=6), and 6 mg/kg JMV 2959-nicotine (n=6).

2.5 Data Analyses

The overall design of this study was a split-plot factorial consisting of the between-group factors of JMV 2959 pre-treatment (0, 3 or 6 mg/kg) and nicotine dose (0 versus 0.4 mg/kg) and a within-group factor of days (8 days: baseline day 0, treatment days 1–7). Statistical significance was deemed to be $p < 0.05$ and the Bonferroni procedure was used to examine significant mean group differences.

3. Results

On the last day of the baseline procedure (Day 0 in panels A–C of Figure 1), there were no significant effects of nicotine dose ($F(1,34) = 0.17$, $P < 0.685$) or of JMV 2959 dose ($F(1,34) = 0.001$, $P < 0.999$), and there was no significant interaction among these factors ($F(2,34) = 0.24$, $P < 0.788$) on baseline 45 min locomotion scores. A split-plot ANOVA of the changes in locomotion induced by 0, 3 or 6 mg/kg JMV 2959 across the treatment days in vehicle treated rats (data depicted in Panels A, B, and C of Figure 1) revealed no significant effect of days ($F(7,119) = 0.556$, $P < 0.790$), or of JMV 2959 dose ($F(2,17) = 1.448$, $P < 0.254$), nor was there a significant interaction between days and JMV 2959 dose ($F(14,119) = 1.055$, $P < 0.405$). Although there was a trend for 6 mg/kg JMV 2959 to reduce locomotion on some days, these differences were not significant nor was there observed an effect of 3 mg/kg JMV 2959 on locomotion in rats treated with vehicle. An overall split-plot ANOVA was computed to determine the impact of nicotine (0 vs. 0.4 mg/kg) and of JMV 2959 (0, 3 or 6 mg/kg) on locomotion across the 8 days of this experiment. These analyses revealed a significant overall effect of day ($F(1,34) = 40.33$, $p < 0.0001$) as well as interactions between nicotine dose and day ($F(1,34) = 48.24$, $p < 0.0001$), JMV 2959 dose and day ($F(2,34) = 9.89$, $p < 0.0001$), and a triple interaction between nicotine dose, JMV 2959 dose and day ($F(2,34) = 4.79$, $p < 0.015$). The latter interaction is depicted in the three panels of Figure 1. In panel A, administration of 0.4 mg/kg nicotine in vehicle pretreated rats initially suppressed locomotion on treatment day 1 and over the next 6 days, this treatment resulted

in significant sensitization of locomotion to levels nearly three times that noted in vehicle treated rats. Post hoc contrasts revealed significant differences ($p < 0.05$) between the Veh/Veh group and the Veh/Nic group on days 5, 6, and 7. In panels B and C, rats pretreated with either 3 or 6 mg/kg JMV 2959 and then treated with nicotine showed an initial (but non-significant) suppression of locomotion lasting 1–2 days, but failed to exhibit a subsequent significant enhancement of locomotion as was induced by 0.4 mg/kg nicotine in panel A. In panels B and C, no contrast between Veh and Nic treatments were significant for any dose of JMV 2959.

4. Discussion

In the present study, we considered the development of locomotor sensitization induced by repeated administration of 0.4 mg/kg nicotine in rats for which GHR receptors were antagonized by pretreatment with the GHR-R antagonist JMV 2959 [30, 32]. Adult male rats pre-treated with JMV 2959 showed significant attenuation of the development of hyperlocomotion to daily injections of 0.4 mg/kg nicotine. JMV 2959 alone produced some reduction of locomotion at 6 mg/kg, but not at 3 mg/kg. This outcome is similar to that noted by Landgren and colleagues (which appeared in print after the submission of this manuscript) in which Long-Evans male rats showed disruption of gross behavior induced by 6 mg/kg JMV 2959, but not by 1, 2 or 3 mg/kg JMV 2959 [43]. In the present study, both 3 and 6 mg/kg JMV 2959 produced similar attenuation of the development of nicotine-induced hyperlocomotion. This profile suggests that the attenuation of nicotine locomotor sensitization was not an artifact of the capacity of JMV 2959 to reduce baseline locomotion. The blunted development of nicotine locomotor sensitization reported herein parallels a recent study from our lab in which the same doses of JMV 2959 were noted to attenuate the sensitization induced by daily injection of 10 mg/kg cocaine in rats (Clifford et al., in press). Taken together, these results strongly implicate functional GHR-R activity as required for the induction of locomotor sensitization by psychostimulants.

As noted earlier, acute pharmacological blockade of GHR-Rs also blocks locomotion, the induction of CPP and the capacity of nicotine to acutely induce dopamine overflow within the nucleus accumbens in mice [34]. These effects are likely to reflect activation by nicotine of cholinergic afferents projecting to the VTA, which in turn activate dopamine overflow within the nucleus accumbens [17]. In the present study, we chose to examine nicotine sensitization in rats, in part because much of the sensitization literature has used rats as subjects [38]. The present study documents that chronic pharmacological inactivation of GHR-Rs substantially diminishes the locomotor sensitization induced by nicotine in rats. In an earlier study, we noted that rats sustaining mutation of the GHR-R resulting in a functional ablation of that receptor do not overeat when given systemic ghrelin injections and show diminished locomotor sensitization to daily injections of cocaine [44]. Similarly, Abizaid and colleagues noted that ghrelin knockout mice exhibit diminished locomotor sensitization to cocaine [45]. Collectively, these studies suggest that GHR-Rs play a prominent role in the development of behavioral sensitization to psychostimulants. Moreover, the present results extend earlier studies in which GHR-Rs were noted to be required for the induction of hyperlocomotion to cocaine, to amphetamine, and to ethanol [8, 22, 26, 33–34].

Inasmuch as GHR-Rs are critically involved in the induction of eating [8, 28, 47], antagonism of GHR-Rs has been a key focus of appetite suppressant drug development. Pharmacological antagonism of GHR-Rs can diminish baseline feeding and attenuate the rewarding action of food [16, 28]. The present study in which antagonism of GHR-R function reduced the development of nicotine sensitization may offer another modality of drug treatment for nicotine addiction. Such an outcome may suggest that GHR-R drug

antagonists may have dual utility for the treatment of nicotine addiction: the first being antagonism of the rewarding action of nicotine; the second being obviation of the weight gain often noted following smoking cessation; such weight gain is often viewed as an important barrier to smoking cessation [48]. Additionally, a distinct haplotype of the GHR-R is associated with smoking risk in low-level female consumers of alcohol [49]. The manner in which this GHSR haplotype alters ghrelin signaling is unknown.

In contrast to the impact of inhibition of GHR signaling on cocaine behavioral function, our earlier laboratory studies showed that administration of GHR facilitates cocaine-induced hyperlocomotion and cocaine-induced CPP [24–25, 27]. It should also be noted that chronic activation of ghrelin receptors using daily administration of ghrelin can induce cross-sensitization to cocaine – suggesting an alteration of the coupling between GHR-Rs and dopamine neurons [25]. This effect may be related to an up-regulation of D1 receptors such that ghrelin can amplify dopamine signaling [50]. Such an outcome is the inverse of that noted in our studies wherein antagonism of GHR-Rs diminished the development of sensitization to nicotine and to cocaine. A more general role for GHR-Rs in brain reinforcement is also indicated by recent studies in which pharmacological inactivation of GHR-Rs attenuates the CPP induced by ethanol [33] and in which genetic ablation of GHR-Rs attenuates the CPP induced by ingestion of a high-fat diet [16]. GHR-Rs also play a key role in the consumption of sweet tastes in rats and humans [51–53]. These converging outcomes strongly support the view that GHR receptors modulate reinforcement/reward function.

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Highlights

- Ghrelin receptor (GHR-R) activity modulates brain reinforcement function.
- Nicotine-induced locomotor sensitization was blocked by the GHR-R antagonist JMV 2959.
- Central ghrelin signaling appears to play a key role in drug reinforcement.

Fig.1.

Mean group total changes in total distance traveled scores (cm/45 minutes). On day 0, the rats were injected with Veh at -5 minutes prior to the 15 minute baseline period and then again with Veh just prior to the 45 minute test period. During days 1–7, the rats were injected with either Veh (panel A), 3 mg/kg JMV 2959 (JMV 3: panel B) or 6 mg/kg JMV 2959 (JMV 6: panel C) at -5 minutes prior to the 15 minute baseline period and then injected with either vehicle or 0.4 mg/kg nicotine (Nic) just prior to the 45 minute test period on days 1–7. The lines above and below each symbol represent the S.E.M. The star (*) indicates a significant ($p < 0.05$) difference between the respective Veh and Nic groups.