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Systemic Ghrelin Sensitizes Cocaine-induced Hyperlocomotion in Rats

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Abstract

The feeding-relevant pathway by which food restriction (FR) augments cocaine action is unknown. Systemic administration of the 28-amino acid acylated peptide ghrelin (1–10 nMol) increases food intake in rats and circulating levels of rat ghrelin are up-regulated by FR. The present experiment examined the impact of repeated administration of ghrelin or vehicle on the subsequent capacity of cocaine to enhance locomotion in rats. Male Sprague-Dawley rats were pretreated daily for seven days with 0, 5 or 10 nMol rat ghrelin (i.p.) in the home cage. On the 8th day, rats were transported to a testing room, placed in a locomotion chamber for 15 min, and then injected (i.p.) with 0, 7.5, or 15 mg/kg cocaine hydrochloride. Locomotor activity was monitored over a 45 min post-cocaine period. Pretreatment with 5 or 10 nMol ghrelin alone did not significantly increase basal locomotion relative to that of the 0 nMol ghrelin group. Rats pretreated with 5 nMol or 10 nMol ghrelin showed an enhanced locomotor response after treatment with 15 mg/kg cocaine relative to rats treated with 0 nMol ghrelin. These results indicate that acute injection of ghrelin, at a feeding-relevant dose, can augment the acute effects of cocaine on locomotion in rats.

Keywords

Food deprivation; Locomotion; Growth Hormone

Food restriction (FR) is known to augment the locomotor actions of psychostimulant drugs such as cocaine or amphetamine (Bell et al., 1997; Campbell and Carroll, 2001; Carr, 2002; Carroll and Meisch, 1980, 1981). Additionally, FR acts to facilitate the acquisition of cocaine or amphetamine self-administration (Carroll, 1985) and augments the acquisition of cocaine-induced conditioned place preference (Bell et al., 1997). When animals already trained to self-administer cocaine are deprived of food, their daily drug intake increases and FR can facilitate the reinstatement of cocaine-seeking behavior (Carroll, 1985). Conversely, food satiation can delay the acquisition of cocaine self-administration (Carroll and Lac, 1998). These findings suggest that feeding and drug addiction may share common pathways (DiLeone et al., 2003).

The neural substrate through which the hyperlocomotor and reinforcing actions of drugs such as cocaine or amphetamine are enhanced by FR remains unknown (Carr, 2002). The stomach peptide ghrelin is an endogenous ligand for the growth-hormone secretagogue receptor (Kojima et al., 1999; Tschop et al., 2000) and functions as an orexigen receptor (Ariyasu et al.,

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2001; Inui et al., 2004; Naleid et al., 2005; Wren et al., 2001a; Wren et al., 2001b). Plasma levels of ghrelin are elevated by FR, whereas administration of ghrelin elicits eating (Ariyasu et al., 2001; Asakawa et al., 2003; Bagnasco et al., 2003; Diano et al., 2006; Gualillo et al., 2002; Inui et al., 2004; Naleid et al., 2005; Tolle et al., 2002; Wren et al., 2001a; Wren et al., 2001b). Ghrelin can gain entry into the CNS (Banks et al., 2002; Diano et al., 2006) and ICV infusions of ghrelin can stimulate locomotion and induce dopamine overflow within the nucleus accumbens (Jerlhag et al., 2006). More importantly, systemic injections of ghrelin (5 nMol/rat) augment the acute locomotor stimulating effects of cocaine (Wellman et al., 2005). In that study, ghrelin was given on four occasions an hour prior to cocaine administration. Given that chronic FR induces a degree of sensitization to cocaine (Bell et al., 1997; Carroll, 1985), it was deemed of interest to determine whether repeated daily (once per day for 7 days) administration of 5 nMol or 10 nMol ghrelin (without cocaine) would result in sensitization to the subsequent hyperlocomotor response to cocaine (**0, 7.5, or 15 mg/kg**) in rats. These doses were chosen on the basis that a 5 nMol ghrelin dose alters cocaine-induced locomotion (Wellman et al., 2005), whereas 10 nMol ghrelin induces the formation of c-Fos-like-immunoreactivity within the hypothalamic paraventricular nucleus (a key structure for the regulation of eating), but not within the area postrema, a region thought to play a role in malaise processes (Ruter et al., 2003). Sensitization to amphetamine, cocaine, and ephedrine can be induced by as few as 7–9 daily doses of cocaine (Horger et al., 1994; Jimenez-Rivera et al., 2006; Miller et al., 1999; Post and Rose, 1976) thus a 7 day pretreatment procedure was used here. With regard to cocaine doses, 7.5 mg/kg is above threshold for induction of locomotion in adult male rats, whereas 15 mg/kg does not induce a maximal locomotor response (Wellman et al., 2002).

Method

Animals

The subjects were 72 adult male Sprague-Dawley rats (Harlan, Houston, TX) weighing 250–275 g at the start of the experiment. The rats were singly housed in hanging polycarbonate rodent cages in a colony room maintained at $23.0 \pm 1^\circ\text{C}$ under a 12 h/12 h illumination schedule (lights off at 1130 h). The experimental procedures and treatments were approved by the Texas A&M University Laboratory Animal Care Committee.

Drugs

Rat ghrelin (Global Peptide, Fort Collins, CO) was dissolved in physiological (0.9% w/v) saline at either a 5 nMol or 10 nMol concentration which was administered intraperitoneally (i.p.) in a fixed volume of 0.5 ml. Cocaine HCl was kindly provided by Dr. Kevin Gormley of the Basic Research Division of NIDA. Cocaine was dissolved in 0.9% w/v saline vehicle in concentrations of 7.5 or 15 mg/ml. All cocaine injections were administered i.p. in volumes of 1 ml/kg body weight.

Apparatus and Procedure

The apparatus consisted of eight optical beam activity monitors (Model RXYZCM-16) and a multiplexor-analyzer (Model DCM-4; Omnitech Electronics, Inc., Columbus, OH). A full description of the apparatus and testing procedures are described in (Miller et al., 1999) On seven consecutive days, each rat was injected (i.p.) with a fixed dose of either 0, 5 or 10 nMol/rat ghrelin and returned to the home cage. On the seventh day of ghrelin pretreatment, the rats were administered saline (1.0 ml/kg, i.p.) and acclimated to the locomotor chambers for 45 min. The final ghrelin pretreatment was given in the home cage just after the adaptation trial. On the eighth day, each rat received one of three cocaine doses (0, 7.5 or 15 mg/kg) and was then placed into an activity chamber for 45 min. Each rat had free access to food and water

(except while in the activity chambers) and activity tests were conducted between 8:00 am and 10:00 am.

Data Analyses

Of the 14 distinct activity measures computed by the Digiscan-16 system, total distance traveled is considered the most appropriate index of general locomotor activity (Miller et al., 1999; Sanberg et al., 1987). The total distance traveled (cm) scores for each 45 min period were integrated using the Versamax software. These measures were subjected to split-plot analyses of variance (ANOVA) using pretreatment (0, 5, or 10 nMol ghrelin) and cocaine dose (0, 7.5, or 10 mg/kg) as between-group factors, whereas session time (0–15, 16–30, and 31–45 min) was used as a within-group factor.

Results

Inasmuch as there was a significant difference between the cocaine groups prior to the cocaine challenge ($F(2,64) = 3.04$, $P < 0.04$), subsequent analyses used the baseline 15 min locomotion scores as a covariate. Pretreatment of the rats with 0, 5, or 10 nMol ghrelin for 7 consecutive days (Fig 1: Panel A) did not alter locomotion scores during the 15 minute period prior to the cocaine challenge ($p = 0.228$). A $3 \times 3 \times 9$ analysis of covariance with the between-group factors of ghrelin dose (0, 5, or 10 nMol) and cocaine dose (0, 7.5 or 15 mg/kg) and the repeated factor of time (every 15 min for 45 min) revealed a significant effect of cocaine dose ($F(2,57) = 28.1$, $p < 0.0001$) and a significant interaction between the factors of cocaine dose, ghrelin dose and time ($F(4,57) = 6.07$, $p < 0.0001$). To explore the interaction between cocaine and ghrelin (Panels B-D), separate 3×3 ANOVAs were computed for each time interval. These analyses indicated a significant interaction between cocaine dose and ghrelin dose for the first 15 min period ($F(4,57) = 2.83$, $p < 0.033$), but not thereafter. Subsequent Bonferroni contrasts indicated that the 15.0 mg/kg cocaine dose produced a significant, **but similar increase**, in total distance traveled scores in the 5.0 and 10.0 nmol ghrelin groups, but not in the 0 nmol ghrelin group.

Discussion

The key finding of the present experiment is that repeated systemic administration of 5 nMol or 10 nMol ghrelin over a seven day period significantly increased the initial effect of subsequent cocaine exposure on locomotion. Specifically, total distance traveled scores after cocaine were elevated during the first 15 min of the 45 min test session in ghrelin pretreated rats. The effect of ghrelin pretreatment was evident at a ghrelin dose of 5 nMol/rat, whereas increasing the dose to 10 nMol/rat did not further increase the augmentation of cocaine-induced locomotion. These findings extend our earlier observation in which the combination of ghrelin and cocaine produced greater locomotion in rats than did cocaine only (Wellman et al., 2005) and suggest that repeated administration of ghrelin can induce a degree of sensitization to cocaine. Although changes in locomotion are not always predictive of abuse potential, it should be noted that rats treated with a combination of ghrelin and cocaine exhibit greater conditioned place preference than do rats treated only with cocaine (Davis et al., 2007) and that changes in plasma ghrelin level induced by a cue previously paired with cocaine self-administration are correlated with reinstatement of responding for cocaine (Tessari et al, 2007).

The capacity of ghrelin to induce a degree of sensitization to cocaine is unlikely to result from activation of peripheral vagal afferents inasmuch as vagotomy does not block the capacity of systemic ghrelin to stimulate eating (Arnold et al., 2006). Systemic ghrelin is passively transported across the blood-brain barrier (Banks et al., 2002; Diano et al., 2006) and can induce the formation c-Fos-like immunoreactivity within the hypothalamus (Ruter et al., 2003). Ghrelin receptors are located on neurons within the arcuate hypothalamus, the hippocampus (Diano et al., 2006) as well as the ventral tegmental area (Naleid et al., 2005) and substantia

nigra (Diano et al., 2006; Guan et al., 1997; Naleid et al., 2005). The localization of ghrelin receptors on ascending dopamine neurons suggests that this neuropeptide may be positioned so as to modulate brain reinforcement circuits. At a cellular level, ghrelin is known to facilitate the impact of dopamine on c-AMP (Jiang et al., 2006). Although Brunetti et al., (Brunetti et al., 2002) reported that bath application of ghrelin was without effect on hypothalamic dopamine release, a recent set of studies in mice indicates that infusions of ghrelin via the third ventricle (Jerlhag et al., 2006) or delivered into the ventral tegmental area (Jerlhag et al., 2007) stimulate locomotion and increase dopamine overflow within the nucleus accumbens. Application of ghrelin does not alter basal firing rates of ventral tegmental area neurons (Korotkova et al., 2006), which suggests that the impact of ghrelin is downstream from the ventral tegmental area.

Another mechanism through which ghrelin may alter the effects of cocaine involves changes in plasma corticosterone related to activation of the HPA axis (Marinelli et al., 1997; Piazza et al., 1998; Rouge-Pont et al., 1995). Stressors, including FR, are known to induce the release of ACTH and corticosterone and induce a degree of sensitization in dopamine signaling pathways (Broocks et al., 1990; Goeders, 2002a; 2002b; Marinelli et al., 1997; Rouge-Pont et al., 1995). ICV administration of ghrelin activates the HPA axis increasing ACTH and corticosterone (Stevanovic et al., 2007; Wren et al., 2001b). In contrast, systemic administration of ghrelin at a dose of 30 nmol does not alter plasma corticosterone (Wren et al., 2001b), suggesting that the changes in cocaine sensitization noted in the current study may not be due to an action of *systemic* ghrelin on corticosterone activity (i.e., in the present study, we used 5 or 10 nmol ghrelin doses – levels that would not be expected to alter systemic corticosterone levels). Moreover, in a recent study (Tessari et al., 2007), food-deprived rats were trained to self-administer cocaine in the context of a CS (tone/light for 5 sec) and then responding was extinguished. On a subsequent trial, reinstatement of responding was evoked by exposure to the CS with serum levels of ghrelin and corticosterone monitored before and after the CS. Plasma ghrelin levels, but not corticosterone levels, were positively related to cue-induced reinstatement of cocaine responding (Tessari et al., 2007). These preliminary studies suggest a modulatory role for ghrelin in cocaine reinforcement and indirectly suggest that corticosterone may not modulate the action of ghrelin on cocaine reinforcement. A final conclusion will await a study in which corticotrophin releasing factor receptor antagonists are used to determine whether ghrelin sensitization can be blocked by antagonism of the HPA axis.

Finally, the present data are consistent with the view that FR may act upon a ghrelin-dependent pathway to augment the psychostimulant action of cocaine. A key test of this hypothesis, however, will come from studies that examine the impact of antagonism of either ghrelin receptors or knockout of ghrelin product to determine whether such manipulations diminish the impact of FR on the psychostimulant action of cocaine (Cummings et al., 2007). It should be noted that ghrelin signaling may be important for psychostimulant-induced reinforcement independent of whether ghrelin is the mediating substrate for FR effects on drug reinforcement.

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Fig 1.

Mean group total distance traveled scores (cm) during 15 min bins prior to (0) or for 45 min (Time bins 1, 2, and 3) for rats treated with 0 mg/kg cocaine and but pretreated with 0, 5 or 10 nmol ghrelin (Panel A), for rats pretreated with 0 nMol ghrelin and then treated with either 0, 7.5, or 15 mg/kg cocaine (Panel B), for rats pretreated with 5 nMol ghrelin and then treated with either 0, 7.5, or 15 mg/kg cocaine (Panel C), or for rats pretreated with 10 nMol ghrelin and then treated with either 0, 7.5, or 15 mg/kg cocaine (Panel D). The lines above each symbol represent the SEM.