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Rhinehart, Erin Keen, and Timothy J. Bartness. Peripheral ghrelin injections stimulate food intake, foraging, and food hoarding in Siberian hamsters. *Am J Physiol Regul Integr Comp Physiol* 288: R716–R722, 2005. First published December 2, 2004; doi:10.1152/ajpregu.00705.2004.—Fasting triggers many effects, including increases in circulating concentrations of ghrelin, a primarily stomach-derived orexigenic hormone. Exogenous ghrelin treatment stimulates food intake, implicating it in fasting-induced increases in feeding, a consummatory ingestive behavior. In Siberian hamsters, fasting also stimulates appetitive ingestive behaviors such as foraging and food hoarding. Therefore, we tested whether systemic ghrelin injections (3, 30, and 200 mg/kg) would stimulate these appetitive behaviors using a running wheel-based food delivery system coupled with simulated burrow housing. We also measured active ghrelin plasma concentrations after exogenous ghrelin treatment and compared them to those associated with fasting. Hamsters had the following: *1*) no running wheel access, free food; *2*) running wheel access, free food; or *3*) foraging requirement (10 revolutions/pellet), no free food. Ghrelin stimulated foraging at 0–1, 2–4, and 4–24 h postinjection but failed to affect wheel running activity not coupled to food. Ghrelin stimulated food intake initially (200–350%, first 4 h) across all groups; however, in hamsters with a foraging requirement, ghrelin also stimulated food intake 4–24 h postinjection (200–250%). Ghrelin stimulated food hoarding 2–72 h postinjection (100–300%), most markedly 2–4 h postinjection in animals lacking a foraging requirement (635%). Fasting increased plasma active ghrelin concentrations in a time-dependent fashion, with the 3- and 30-mg/kg dose creating concentrations of the peptide comparable to those induced by 24–48 h of fasting. Collectively, these data suggest that exogenous ghrelin, similar to fasting, increases appetitive behaviors (foraging, hoarding) by Siberian hamsters, but dissimilar to fasting in this species, stimulates food intake.

enzyme-linked immunosorbant assay; appetitive; consummatory; feeding

OBESITY IS AN EPIDEMIC of literally and figuratively growing proportions. Determining the physiological factors that regulate ingestive behavior is critical to understanding the etiology of obesity. Ingestive behavior occurs in two phases: *1*) the actual eating of the food or the "consummatory" phase and *2*) the acquisition and storage of food or the "appetitive" phase (14). The consummatory aspects of ingestive behavior have received almost exclusive attention in the quest to understand the mechanisms underlying food intake. As for the appetitive phase of ingestive behavior, however, there is comparatively little known about the mechanisms underlying the search for food or "foraging," which is surprising, given its pervasive nature across animal taxa. Perhaps this is because of the difficulty in conducting field studies of foraging or the problem of creating a laboratory-based analog of this behavior. In addition, "food hoarding," the storage of food for later ingestion, is another appetitive ingestive behavior with widespread expression among animal species (for review, see Ref. 47), but studies of the mechanisms underlying this appetitive ingestive behavior have received even less attention than foraging (for review, see Ref. 7).

Siberian hamsters (*Phodopus sungorus*) and other hamster species (for review, see Ref. 8) either do not respond to the energetic challenge of food deprivation by increasing food intake or show relatively small and short-lived increases in food intake after a fast. Instead, Siberian hamsters markedly increase foraging (7, 16) and food hoarding (5, 6, 50). Siberian hamsters and many other animals (for review, see Ref. 7) use food hoarding as a crucial part of their ingestive behavior repertoire in response to many energetic challenges (e.g., pregnancy and lactation; Refs. 5, 7). The physiological mechanism underlying the control of these appetitive ingestive behaviors is largely unknown. To begin to uncover these mechanisms, we developed a simulated burrow housing system in the laboratory (6) to study food hoarding and recently married it to the wheel running-based foraging model of Perrigo and Bronson (35), yielding two important characteristics of foraging and hoarding in natural settings: increased effort and distance to obtain food source. Thus this wheel running-based foraging/hoarding system allows the study of both appetitive and consummatory ingestive behaviors. Studying Siberian hamsters affords additional advantages, because foraging and food hoarding in this species are typically uncoupled from one another (i.e., both do not necessarily increase or decrease together, e.g., fasting; Refs. 5, 6, 9, 16) unlike laboratory rats (e.g., Refs. 33, 43).

We recently began determining the roles of peptides in the control of foraging, food intake, and food hoarding using Siberian hamsters and our foraging/hoarding system by testing the effects of centrally administered agouti-related protein (AgRP; Ref. 17) and neuropeptide Y (NPY; D. E. Day and T. J. Bartness, unpublished results). Both AgRP and NPY strikingly increase food hoarding more than food intake (17, D. E. Day and T. J. Bartness, unpublished results). One neural response to fasting is increases in arcuate nucleus NPY and AgRP gene expression occurring in Siberian hamsters (29, 30) and other species such as laboratory rats and mice (11, 25, 31, 32). These changes, together with their stimulation of foraging

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and food hoarding (17; D. E. Day and T. J. Bartness, unpublished results), suggest the possible involvement of NPY and AgRP in the fasting-induced increases in these appetitive behaviors. This begs the question as to the nature of the fasting-associated stimulus that triggers the increased expression and presumably release of these peptides. One possibility is the fasting-induced, increased release of ghrelin (e.g., Ref. 44) into the circulation from the stomach.

Ghrelin is a 28-amino acid peptide produced primarily in the oxyntic glands of the stomach (2, 3, 26). Ghrelin has been implicated in meal initiation both in rodents and humans because circulating ghrelin concentrations increase preprandially, decrease postprandially, and increase with fasting (2, 44, 46). Thus it is possible that ghrelin serves as a peripherally generated signal to these central peptidergic effectors to stimulate ingestive behaviors (4, 20, 24). Siberian hamsters primarily increase appetitive ingestive behaviors after a fast, perhaps via increases in the orexigenic neuropeptides NPY and AgRP (17; D. E. Day and T. J. Bartness, unpublished results). In addition, ghrelin administered systemically (3, 39, 45) or centrally (3, 4, 19, 34, 53) stimulates food intake, body mass gain, and adiposity in rodents and in humans and increases hunger in humans (51). Together, these data suggest a potential role for ghrelin not only in the consummatory ingestive behaviors studied to date but also in the less frequently investigated appetitive ingestive behaviors such as foraging and food hoarding.

Therefore, the purpose of the present experiments was to test whether peripherally administered ghrelin would stimulate the appetitive ingestive behaviors of foraging and food hoarding, perhaps in an analogous manner to that occurring after fasting. This was accomplished using Siberian hamsters and our foraging/hoarding system to determine the effects of peripheral ghrelin treatment on appetitive and consummatory ingestive behaviors. In addition, we tested whether the exogenously administered ghrelin produced circulating concentrations of the active form of ghrelin within the physiological range associated with fasting in this species.

METHODS

Animals

All procedures were approved by the Georgia State University Institutional Animal Care and Use Committee and are in accordance with Public Health Service and United States Department of Agriculture guidelines. Adult male Siberian hamsters ~3.5 mo old and weighing 35–43 g were obtained from our breeding colony. The lineage of this colony has been described recently (15). Hamsters were group housed and raised in a long-day photoperiod (16:8-h light-dark cycle, lights on at 0200) from birth. Room temperature was maintained at 21 ± 2.0°C.

Hamsters were acclimated for 2 wk in specially designed hoarding apparatuses as previously described (15). Briefly, two cages were connected with a convoluted polyvinylchloride tubing system (38.1 mm ID and ~1.52 m long) with corners and straightways for horizontal and vertical climbs. The top or "food cage" was 456 mm × 234 mm × 200 mm (length × width × height) and was equipped with a water bottle. The bottom or "burrow cage" was 290 mm × 180 mm × 130 mm (length × width × height), was covered to simulate the darkness of a natural burrow, and contained bedding and cotton nesting material. The test diet (75-mg pellets, Purified Rodent Diet; Research Diets, New Brunswick, NJ) and tap water were available ad libitum during this period. A running wheel (524 mm in circumference) and pellet dispenser were attached to the food (*top*) cage. Wheel revolutions were counted with a magnetic detection system and monitored by a computer-based hardware-software program (Med Associates, Lancaster, NH).

Measurement of Foraging, Food Hoarding, and Food Intake

Foraging (pellets earned) was defined as the number of pellets delivered upon completion of 10 wheel revolutions. Food hoarding (pellets hoarded) was defined as the number of pellets found in the bottom burrow cage in addition to those removed from the cheek pouches. Surplus pellets were defined as the number of pellets removed from the *top* cage that were neither eaten nor hoarded. For the 10-revolutions/pellet group, food intake (pellets eaten) was defined as, pellets earned − surplus pellets − hoarded pellets = food intake. For the free and blocked wheel groups, food intake (pellets eaten) was defined as, pellets given − surplus pellets − hoarded pellets = food intake.

Foraging Training Regimen

We used a wheel running training regimen that eases the hamsters into the foraging effort without large changes in body mass or food intake (15). Specifically, all hamsters were given free access to food pellets for 3 days while they adapted to the running wheel. In addition to the free food, a 75-mg food pellet was dispensed upon completion of every 10 wheel revolutions. On the fourth day, the free food condition was replaced by a response-contingent condition, where only every 10 wheel revolutions triggered the delivery of a food pellet. This condition was in effect for 5 days, during which time body mass, food intake, food hoarding, wheel revolution, and pellets earned were measured daily.

Experimental Design

At the end of the training period, hamsters were separated into three groups matched for body weight and average hoard size across the last 3 days of retraining at 10 revolutions/pellet (n = 14/group). The three groups consisted of the following: *1*) 10 revolutions group, 10 wheel revolutions/pellet; *2*) free wheel group, noncontingent food, running wheel access (exercise and nonforaging control); and *3*) blocked wheel group, noncontingent food, immobile running wheel (sedentary and nonforaging control). Each group received all doses of ghrelin and saline, and injections were given in a counterbalanced schedule to control for possible order effects of peptide administration.

Experiment 1: does peripheral ghrelin stimulate appetitive and consummatory behaviors in Siberian hamsters? Forty-two male hamsters were trained in the foraging apparatus as described above. They were separated into the three groups listed above (n = 14 each group) matched on baseline hoarding levels and absolute body mass. Three doses of rat ghrelin (Bachem Bioscience, King of Prussia, PA) dissolved in sterile vehicle (0.15 M NaCl) were chosen on the basis of doses used for other rodents in the literature (3.0, 30, and 200 mg/kg body mass; Refs. 3, 39, 45).

A within-subjects design was chosen to limit variability; therefore, all animals received all doses of ghrelin as well as saline. On the basis of pilot data, there were no carryover effects of ghrelin beyond 7 days; therefore, a washout period of 7 days occurred between the counterbalanced injections until each animal had received each dose of ghrelin and the saline vehicle control. Food intake, food hoarding, foraging, and wheel running were measured at 1, 2, 4, and 24 h and every 24 h for 7 days postinjection.

Experiment 2: are the exogenous ghrelin injection-induced plasma ghrelin concentrations comparable with those generated by fasting? Thirty male hamsters were obtained from our breeding colony and separated into five groups (n = 6/group). One group was fasted for 48 h, with blood samples taken at 0, 12, 24, 36, and 48 h from the start of the fast. The other four groups were injected with the saline vehicle

or one of three doses of ghrelin (3.0, 30, or 200 mg/kg), and blood samples were taken at 0, 1.5, 4, 24, and 48 h. All blood samples were taken from the orbital sinus of animals anesthetized with isoflurane.

Blood Samples

Whole blood samples were drawn directly into heparinized microtainer tubes containing EDTA-2Na (Becton Dickinson, Franklin Lakes, NJ) and 500 U aprotinin (MP Biomedicals, formerly ICN Biomedicals, Aurora, OH). The tubes were then centrifuged at 1,500 *g* for 20 min at 4°C. Immediately after plasma collection, 100 μl of 1 N HCl were added per milliliter of the plasma sample. Plasma was then stored at −80°C until use in the active ghrelin ELISA.

Plasma Active Ghrelin ELISA

Plasma active ghrelin concentrations were measured using a commercial ELISA kit for the active form of ghrelin (Linco Research, St. Charles, MO) according to manufacturer's specifications. Briefly, all reagents were warmed to room temperature before use and diluted or reconstituted, as directed. Assay buffer and 50 μl of sample were placed in the wells of the microtiter plate, coated with mouse monoclonal antibodies to the NH_2-terminus of active ghrelin. The plate was sealed and incubated at room temperature for 2 h. Wells were then washed with buffer three times, after which solutions were decanted and the horseradish peroxidase-labeled mouse monoclonal antibody (1:100) to the COOH terminus of active ghrelin was added. The plate was sealed and incubated at room temperature for 1 h. Next, all wells were washed four times with wash buffer, the substrate solution (3,3′-5,5′-tetramethylbenzidine) was added, and the plate was sealed and incubated in the dark for 30 min. Finally, stop reagent was added, the plate was shaken, and the absorbance was read at 450 nm immediately.

Statistics

For *experiment 1*, the food intake, food hoarding, and foraging data were analyzed using a two-way ANOVA (time × drug), and Bonferroni post hoc test was used for individual pairwise comparisons. For *experiment 2*, data also were analyzed with a two-way ANOVA (time × drug) with the Bonferroni posttest for individual differences for animals given ghrelin, whereas a one-way ANOVA was used for the fasted animals. Differences between means were considered statistically significant if $P < 0.05$. Exact probabilities and test values were omitted for simplicity and clarity of the presentation of the results.

RESULTS

Experiment 1: Does Peripheral Ghrelin Treatment Stimulate Appetitive and Consummatory Behaviors in Siberian Hamsters?

Wheel running. There was no effect of any dose of ghrelin treatment on wheel running activity that was not coupled to food availability, as seen by the free wheel group of hamsters that had food available noncontingently (data not shown). This suggests that there was not general stimulation nor inhibition of locomotor activity by the peptide.

Foraging. The highest dose of ghrelin (200 mg/kg) significantly stimulated wheel running activity to obtain food (foraging, 10 revolutions/pellet) only at the first time point (0–1 h, $P < 0.05$; Fig. 1). The 3- and 30-mg/kg doses of ghrelin similarly significantly stimulated foraging ~150–300% above saline at the 0–1, 2–4, and 4–24 h time points ($P < 0.05$; Fig. 1). No dose of peripheral ghrelin stimulated foraging at the 24–48 or 48–72 h time points.

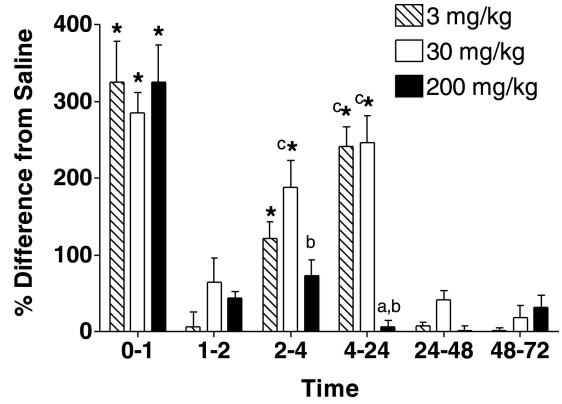

Fig. 1. Mean ± SE foraging (pellets earned) as a percentage of the saline-injected controls for the effects of peripheral ghrelin treatment on hamsters with foraging requirements (10 revolutions/pellet group). *$P < 0.05$ compared with saline injection, [a]$P < 0.05$ compared with the low dose (3.0 mg/kg), [b]$P < 0.05$ compared with the middle dose (30 mg/kg), and [c]$P < 0.05$ compared with the high dose (200 mg/kg) of ghrelin.

Food intake. All doses of ghrelin significantly stimulated food intake to a similar degree (~200–350%) at the 0–1 h period for all groups, regardless of wheel condition, compared with saline injection ($P < 0.05$; Fig. 2). Ghrelin treatment significantly stimulated food intake at the 1–2 and 2–4 h time intervals in an approximately dose-dependent manner for both nonforaging groups (blocked wheel and free wheel, $P < 0.05$; Fig. 2), although the magnitude of stimulation was not as great as that occurred at the 0–1 h time interval. Unlike the nonforaging groups, the foraging group (10 revolutions/pellet) significantly increased food intake 4–24 h postinjection at the 3- and 30-mg/kg ghrelin doses ($P < 0.05$; Fig. 2). No dose of ghrelin stimulated food intake at any time after 24 h postinjection for any group.

Food hoarding. Ghrelin significantly stimulated food hoarding beginning at 2–4 h postinjection for all but the 3-mg/kg dose in the free wheel and blocked wheel groups ($P < 0.05$; Fig. 3). Foraging hamsters (10 revolutions/pellet) significantly increased food hoarding ~200–600% from 2 h postinjection through *day 5* postinjection for all doses of ghrelin and at all time intervals with a few exceptions: the 3- and 200-mg/kg doses 2–4 h postinjection and the all doses 48–72 h postinjection. Hamsters in the free wheel group significantly increased food hoarding to the greatest extent (~400–635%) after 30 mg/kg ghrelin at 2–4 and 4–24 h postinjection. During the 48–72 h time interval, however, all doses produced a similar ~200–300% increase in food hoarding by these hamsters compared with saline. Interestingly, there was no effect of any dose of ghrelin on food hoarding in the free wheel group at *day 4* postinjection; however, the 3- and 30-mg/kg doses significantly stimulated food hoarding on *day 5* postinjection (250–300%, $P < 0.05$; Fig. 3). As with the free wheel group, the 30-mg/kg dose of ghrelin was the most effective at stimulating food hoarding behavior (200–300%) in the blocked wheel group. Ghrelin treatment appeared to have less of an effect on food hoarding in the blocked wheel group compared with the free wheel and 10 revolutions/pellet groups, because the largest increase in the blocked wheel group was ~300%, unlike both the wheel running groups that showed increases of 600% or more at some time interval.

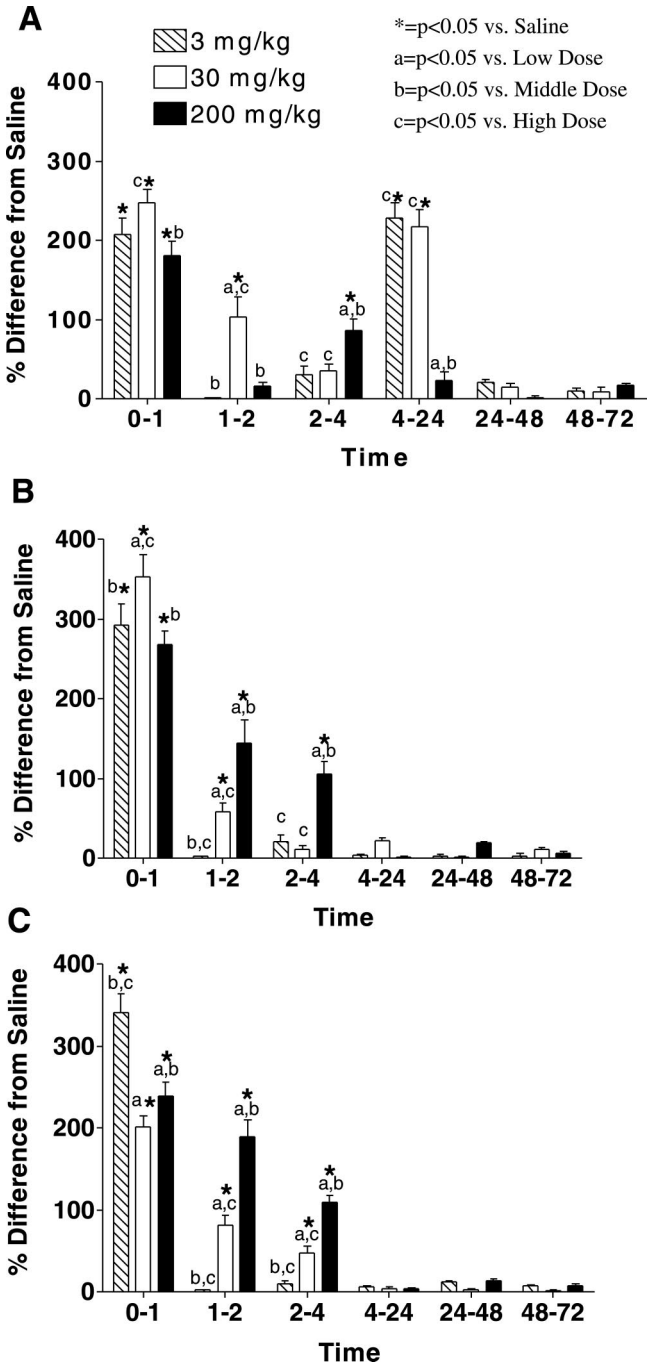

Fig. 2. Mean ± SE food intake (pellets eaten) as a percentage of the saline-injected controls for the effects of peripheral ghrelin treatment. *A*: hamsters with a foraging requirement of 10 revolutions/food pellet. *B*: hamsters with no foraging requirement but a freely moving wheel (free wheel group). *C*: hamsters with no foraging requirement and no moving wheel (blocked wheel group). *$P < 0.05$ compared with saline injection, [a]$P < 0.05$ compared with the low dose (3.0 mg/kg), [b]$P < 0.05$ compared with the middle dose (30 mg/kg), and [c]$P < 0.05$ compared with the high dose (200 mg/kg) of ghrelin.

Experiment 2: Are the Exogenous Ghrelin Injection-Induced Plasma Ghrelin Concentrations Comparable with Those Generated by Fasting?

The doses of ghrelin used in this study were chosen based on those used in laboratory rats (3, 39, 45). To determine whether these doses created circulating active ghrelin concentrations in the physiological range of those seen during mild to moderate fasting in Siberian hamsters, we fasted hamsters or treated them with peripheral ghrelin injections and sampled blood at several time points postmanipulation.

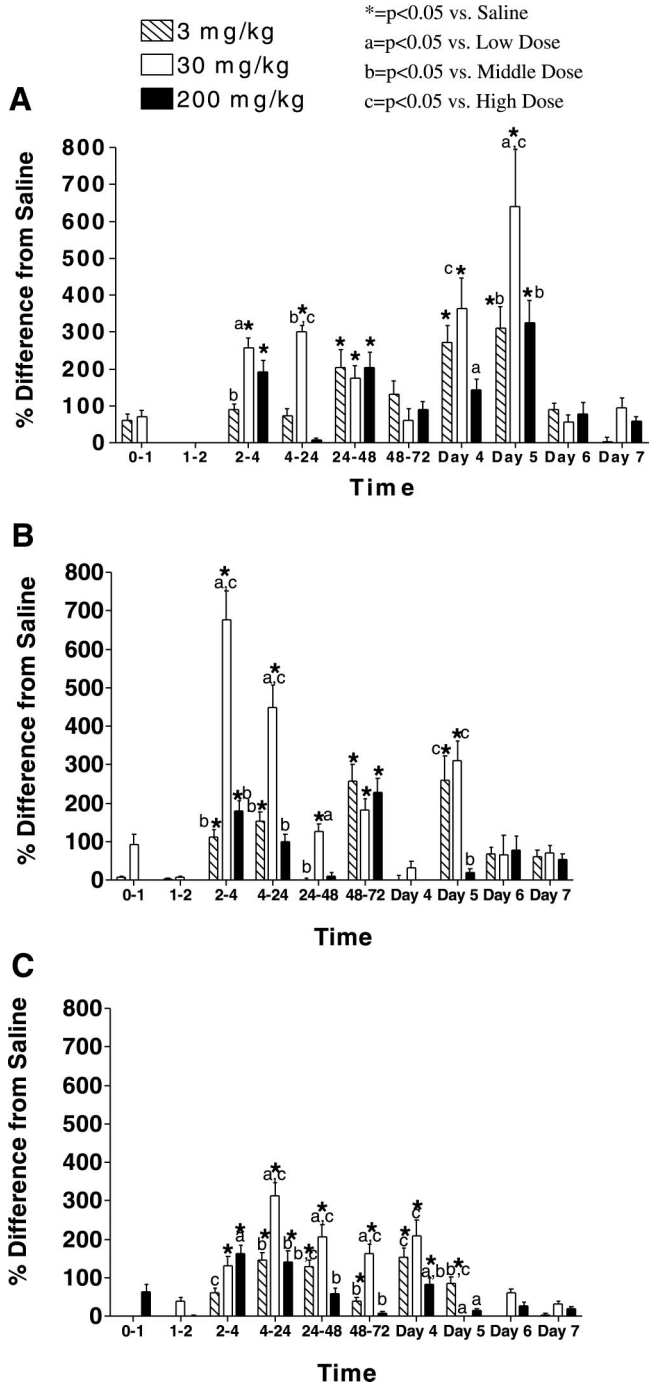

Fig. 3. Mean ± SE food hoarding (pellets hoarded) as a percentage of the saline-injected controls for the effects of peripheral ghrelin treatment. *A*: hamsters with a foraging requirement of 10 revolutions/food pellet. *B*: hamsters with no foraging requirement but a freely moving wheel (free wheel group). *C*: hamsters with no foraging requirement and no moving wheel (blocked wheel group). *$P < 0.05$ compared with saline injection, [a]$P < 0.05$ compared with the low dose (3.0 mg/kg), [b]$P < 0.05$ compared with the middle dose (30 mg/kg), and [c]$P < 0.05$ compared with the high dose (200 mg/kg) of ghrelin.

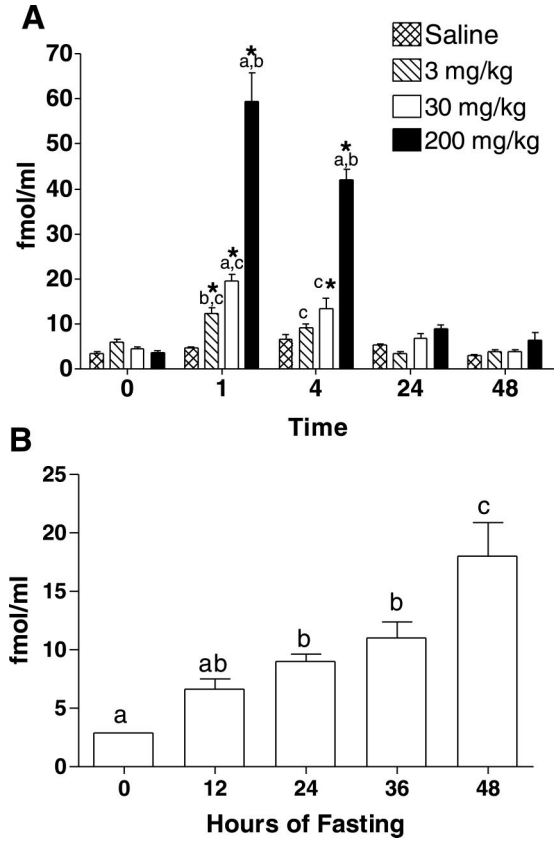

Fig. 4. *A*: effects of ghrelin treatment on plasma active ghrelin concentrations at time points from 0 to 72 h postinjection. $^{*}P < 0.05$ compared with saline injection, $^{a}P < 0.05$ compared with the low dose (3.0 mg/kg), $^{b}P < 0.05$ compared with the middle dose (30 mg/kg), and $^{c}P < 0.05$ compared with the high dose (200 mg/kg) of ghrelin. *B*: 12-h time points of 0–48 h of fasting. $P < 0.05$ for different letters.

The lowest dose of ghrelin (3.0 mg/kg) created active ghrelin plasma concentrations similar to those in animals undergoing 24–36 h of fasting at 1.5 h postinjection (Fig. 4). The middle dose of ghrelin (30 mg/kg) caused active ghrelin concentrations in plasma that were similar to 36–48 h of fasting at 1.5 h postinjection and active ghrelin plasma concentrations similar to 24 h of fasting at 4–24 h postinjection (Fig. 4). The highest dose of ghrelin (200 mg/kg) generated supraphysiological active ghrelin plasma concentrations that were nearly three times greater than those exhibited by hamsters fasted for 48 h. All animals given ghrelin injections exhibited declining active ghrelin concentrations in plasma in a time-dependent fashion, with concentrations returning to baseline levels 24 h postinjection.

DISCUSSION

The physiological mechanisms regulating appetitive behaviors are not well understood. Insight into how peripheral factors such as ghrelin affect central neural circuits regulating both appetitive and consummatory behaviors is critical to a full understanding of the regulation and dysregulation (obesity) of energy homeostasis. The results of the present study suggest that ghrelin has additional roles beyond its apparent involvement in meal initiation (2, 46, 51), showing for the first time that ghrelin also is involved in appetitive ingestive behaviors. Specifically, peripherally administered ghrelin that generated circulating concentrations of the active peptide in the range generated naturally by 24–48 h of fasting significantly increased both the appetitive ingestive behaviors of foraging and food hoarding as well as the consummatory behavior of food intake. Thus these results demonstrate a new function of ghrelin, stimulation of foraging and food hoarding, at least in Siberian hamsters.

The stimulation of foraging by ghrelin appears to be a genuine effect of the peptide on the effort to acquire food, because wheel running per se was not significantly increased by any dose of ghrelin. The stimulation of foraging was evident early (0–1 h postinjection) and occurred for all doses of ghrelin; however, there was a marked period of decreased wheel running 1–2 h postinjection, as seen in decreased foraging by the 10 revolutions/pellet group, as well as decreased wheel running per se by the free wheel group. During this period, food intake, but not food hoarding, was significantly increased in both of these groups (10 revolutions/pellet at 30 mg/kg, ~100% increase; free wheel at 30 and 200 mg/kg, ~75–150% increase). Although we did not directly observe these animals across the test, it is unclear as to what behaviors beyond increased feeding occurred during this interval. To our knowledge, this is the first report of a peripheral peptide increasing foraging in any species.

The effects of ghrelin on food intake were relatively short lived, being present only during the initial 4 h postinjection, except for the hamsters foraging for their food (10 revolutions/pellet), where food intake also was significantly increased 4–24 h postinjection. The short duration of ghrelin-stimulated food intake is also seen with centrally or peripherally administered ghrelin in laboratory rats (23, 45, 53) and mice (3, 45). The temporal pattern of ghrelin-induced increased food intake in the present experiment paralleled that of the elevation of active ghrelin concentrations in plasma for the nonforaging hamsters (free wheel and blocked wheel groups), being significantly elevated at the two highest doses (30 and 200 mg/kg) for up to 4 h postinjection. Hamsters required to forage for their food (10 revolutions/pellet), however, had a second significant increase in food intake 4–24 h postinjection for the two lowest doses of ghrelin (3 and 30 mg/kg) in addition to their significantly increased food intake across the first 4 h postinjection (0–1 h, all three doses; 1–2 h, 30 mg/kg; 2–4 h, 200 mg/kg). Because there is no evidence to indicate the continued presence of circulating active ghrelin much after 4 h postinjection, this second bout of food intake seems likely to be due to effects of the peptide on downstream pathways from one of ghrelin's central targets. Likely targets are the arcuate nucleus NPY/AgRP neurons, given the ability of ghrelin to activate these neurons producing orexigenic peptides (28) and given the lingering effects of centrally administered AgRP on food intake in this species (17). The second surge in food intake by the foraging hamsters also could be due to simple behavioral competition, as the hamsters were engaging in both appetitive ingestive behaviors (foraging, hoarding) during the period between the two bouts of food intake. These data further amplify our previous conclusion (17; D. E. Day and T. J. Bartness, unpublished results) and that of others (42) suggesting that the effects of centrally acting peptides on food intake can be modified by environmental factors, such as requiring an explicit appetitive response.

Ghrelin stimulated foraging and food hoarding within 2–4 h postinjection regardless of foraging requirement (~100–650%). The marked ghrelin-induced increase in food hoarding persisted up to 5 days postinjection, similar to the length of increased food hoarding caused by centrally administered AgRP in this species (17). As for the food intake discussed above, because the significantly elevated active ghrelin plasma concentrations had returned to baseline values by at least 24 h postinjection, it is likely that postreceptor events triggered the enduring effects of the peptide on food hoarding. It seems more than coincidental that ghrelin stimulates arcuate NPY/AgRP neurons (13, 23, 24) and that intracerebroventricular injections of AgRP also produce persisting stimulation of food hoarding within the same timeframe (17); therefore, at present, this seems the most likely mechanism producing this effect. It still remains a biological mystery, however, as to the mechanisms underlying the enduring AgRP stimulation of food hoarding in this species (17), as well as the similar lingering effects of AgRP-induced increased food intake by laboratory rats (21, 22). Alternatively, the long-lasting stimulation of food hoarding by ghrelin could be via an unrelated mechanism, and, if so, then this would be a second example of persisting effects of single peptide administrations on ingestive behaviors.

The doses of ghrelin given in this study were based on studies using laboratory rats (39, 45, 52, 53). In general, hamster species (Syrian or Siberian) appear to be less sensitive or completely insensitive to many of the substances that reliably increase ingestive behavior in laboratory rats [e.g., 2-deoxy-D-glucose (1, 6, 36–38, 41) compared with NPY (10, 12, 27)]. Therefore, we deemed it necessary to test several doses of ghrelin and to document the consequent changes in active ghrelin plasma concentrations. Moreover, it was important to test whether any of these ghrelin doses produced active ghrelin plasma concentrations within the physiological range of fasted hamsters. Clearly, the highest dose used here (200 mg/kg) was supraphysiological, resulting in active ghrelin plasma concentrations at least three times that seen in the longest duration of fasting (48 h). The other two doses of ghrelin (3 and 30 mg/kg), however, generated active ghrelin plasma concentrations similar to those observed in Siberian hamsters fasting for 24–48 h, at least 1.5 and 4 h postinjection, times when ghrelin increased foraging, food hoarding, and food intake in these animals. Thus one could argue that the marked increased appetitive and consummatory ingestive behaviors triggered by peripherally administered ghrelin in the present study were due to creating elevated circulating active ghrelin concentrations in the physiological range, perhaps mimicking the effects of fasting-induced increased plasma ghrelin on these two classes of ingestive behaviors.

Collectively, the results of this study, combined with known relations among fasting, ghrelin, NPY/AgRP arcuate nucleus neurons, and food hoarding as reviewed above, suggest the following scenario. First, fasting induces increases in active ghrelin in plasma (Refs. 2, 44 and the present experiment) that, in turn, stimulate arcuate nucleus NPY/AgRP synthesizing neurons (13, 24, 48). Release of AgRP and NPY in the paraventricular nucleus of the hypothalamus, and perhaps other areas, stimulates circuits underlying the postfast increases in food hoarding and food intake in this species (29, 40, 49). The persisting stimulation of food hoarding may be due to AgRP-activated circuits, given that NPY administration does not produce long-lasting (>24 h) increases in food hoarding (D. E. Day and T. J. Bartness, unpublished observations). This sequence of events clearly requires substantial subsequent testing.

In summary, ghrelin appears important for both appetitive and consummatory behaviors in Siberian hamsters. Ghrelin stimulation of consummatory behavior was immediate but short lived, whereas ghrelin stimulation of appetitive behaviors was typically delayed and long lasting. It is likely that different central mechanisms are responsible for these different behavioral sequences because of their separation in time and duration.

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GRANTS

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