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INHIBITION OF GHRELIN O-ACYLTRANSFERASE ATTENUATES FOOD DEPRIVATION-INDUCED INCREASES IN INGESTIVE BEHAVIOR¹

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Abstract

Ghrelin is an orexigenic hormone produced by the stomach in direct proportion to the time since the last meal and has therefore been called a 'hunger signal'. The octanoylation of ghrelin is critical for its orexigenic functions and is dependent upon ghrelin O-acyltransferase (GOAT) catalyzation. The GOAT inhibitor, GO-CoA-Tat, decreases the circulating concentrations of octanoylated ghrelin and attenuates weight gain on a high fat diet in mice. Unlike rats and mice, Siberian hamsters and humans do not increase food intake after food deprivation, but increase food hoarding after food deprivation. In Siberian hamsters, exogenous ghrelin increases ingestive behaviors similarly to 48–56 h food deprivation. Therefore, we tested the necessity of increased ghrelin in food-deprived Siberian hamsters to stimulate ingestive behaviors. To do so we used our simulated natural housing system that allows hamsters to forage for and hoard food. Animals were given an injection of GO-CoA-Tat (i.p., 11 μ mol/kg) every 6 h because that is the duration of its effective inhibition of octanoylated ghrelin concentrations during a 48 h food deprivation. We found that GO-CoA-Tat attenuated food foraging (0–1 h), food intake (0–1 and 2–4 h), and food hoarding (0–1 h and 2 and 3 d) post-refeeding compared with saline treated animals. This suggests that increased octanoylated ghrelin concentrations play a role in the food deprivation-induced increases in ingestive behavior. Therefore, ghrelin is a critical aspect of the multi-faceted mechanisms that stimulate ingestive behaviors, and might be a critical point for a successful clinical intervention scheme in humans.

Keywords

hoarding; food intake; foraging; Siberian hamster; body mass

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Introduction

Obesity is a critical health problem in industrialized nations (Calle, Rodriguez, Walker-Thurmond, and Thun, 2003; Goldstein, Bushnell, Adams, Appel, Braun, Chaturvedi, Creager, Culebras, Eckel, Hart, Hinchey, Howard, Jauch, Levine, Meschia, Moore, Nixon, and Pearson, 2011; Harvey, Lashinger, and Hursting, 2011; Rao, Thethi, and Fareed, 2011; Reaven, 2011; Vague, Vague, Tramon, Viallettes, and Mercier, 1980; Zalesin, Franklin, Miller, Peterson, and McCullough, 2011) because of its multiple associated secondary health consequences, including stroke, some cancers, heart disease, and Type II diabetes. As the prevalence of obesity grows, so does its burden upon the health care system, an estimated \$147 billion was spent on obesity/related disorders in the USA in 2008 (Finkelstein, Trogon, Cohen, and Dietz, 2009). Therefore, the prevention/reversal of obesity is of critical health and financial importance. In its most simple conceptualization, obesity arises when energy intake is greater than energy expenditure, and reducing and/or increasing energy intake and/or expenditure, respectively, is paramount in preventing/reversing obesity. Contributing factors to overconsumption that are often overlooked are the increased prevalence of calorically dense foods/drinks, their long shelf-lives, and increased storage capacity for these items [for review: (Bartness, Keen-Rhinehart, Dailey, and Teubner, 2011)].

In 1918, Wallace Craig dichotomized goal oriented behaviors into “appetitive” (the steps leading to the realization of the goal) and “consummatory” (the actual consummation of the behavior) phases (Craig, 1918). Ingestive behavior can be divided accordingly into these categories, appetitive: food foraging and food hoarding, and consummatory: food intake. Most ingestive behavior research focuses on the consummatory phase of the complex behavioral sequence that results in food intake and this research has contributed invaluable information about ingestive behavior. By contrast, a relatively small amount of research examines the appetitive phase of ingestive behavior [for review: (Bartness *et al.*, 2011)]. In order to test this vital suite of ingestive behaviors, as food cannot be ingested unless it is sought and obtained, we have used Siberian hamsters because, like humans [for review: (Bartness *et al.*, 2011)], and unlike laboratory rats and mice (Calhoun, 1962; Hill, Fried, and DiGirolamo, 1984; Takahashi and Lore, 1980), they hoard food in nature (Flint, 1966), a behavior that can be duplicated in the laboratory (Masuda and Oishi, 1988; Wood and Bartness, 1996). Thus, using our simulated burrow system and wheel-running based food pellet delivery system we have a model system to study both appetitive (foraging and hoarding) and consummatory ingestive behaviors [full description below; (Keen-Rhinehart and Bartness, 2005)].

Food deprivation is a potent stimulator of ingestive behaviors. Siberian hamsters and humans are similar in their response to food deprivation in that both overhoard [for review: (Bartness *et al.*, 2011)], as opposed to overeating like laboratory rats and mice (Hill *et al.*, 1984; Williams and Campbell, 1961; Zhao and Cao, 2009). During food deprivation or between meals, ghrelin, a gut derived peptide, is released into circulation in direct proportion to the time human or non-human animals last ate (Ariyasu, Takaya, Tagami, Ogawa, Hosoda, Akamizu, Suda, Koh, Natsui, Toyooka, Shirakami, Usui, Shimatsu, Doi, Hosoda, Kojima, Kangawa, and Nakao, 2001; Cummings, Purnell, Frayo, Schmidova, Wisse, and Weigle, 2001; Kojima, Hosoda, Date, Nakazato, Matsuo, and Kangawa, 1999; Tschop, Smiley, and Heiman, 2000), including Siberian hamsters (Keen-Rhinehart *et al.*, 2005).

Circulating ghrelin exists in two major forms, des-n-octanoyl ghrelin (des-acyl ghrelin) and octanoylated ghrelin (acyl ghrelin). The acylation of des-acyl ghrelin is critical for it to bind to its receptor, growth hormone secretagogue receptor 1a (GHSR), and is catalyzed by

ghrelin *O*-acyltransferase [GOAT; (Gutierrez, Solenberg, Perkins, Willency, Knierman, Jin, Witcher, Luo, Onyia, and Hale, 2008; Yang, Brown, Liang, Grishin, and Goldstein, 2008)]. Exogenous acyl ghrelin administration increases food intake and body weight in laboratory rats and mice (Nakazato, Murakami, Date, Kojima, Matsuo, Kangawa, and Matsukura, 2001; Tschoep *et al.*, 2000; Wren, Small, Ward, Murphy, Dakin, Taheri, Kennedy, Roberts, Morgan, Ghatei, and Bloom, 2000), findings that have been extended to food foraging and hoarding in Siberian hamsters using our foraging and hoarding apparatus (Keen-Rhinehart *et al.*, 2005). Ghrelin has been termed the “hunger signal” due to the conditions of its natural release and its ability to increase food intake when given exogenously; however, when ghrelin, GOAT, or GHSR is genetically eliminated, as in GHR KO, GOAT KO, and GHSR KO mice, the predicted deficits in food intake and low body weight are not found under most conditions [for review: (Kang, Zmuda, and Sleeman, 2011)]. For example, GOAT KO mice exhibited decreased hedonic feeding and operant responding after food deprivation (Davis, Perello, Choi, Magrisso, Kirchner, Pfluger, Tschoep, Zigman, and Benoit, 2012). Even if the KO mice had shown feeding behavior changes, genetic manipulations are not readily available in Siberian hamsters, therefore leaving us unable to directly test the effect of genetic deletion on food foraging and food hoarding. A recently developed compound inhibits GOAT activity (GO-CoA-Tat). GO-CoA-Tat is a synthetic peptide-coenzyme A conjugate which was designed as a bisubstrate analog for GOAT inhibition. Prior studies showed it to be a selective GOAT inhibitor in vitro, in cell culture, and in mice), consequently decreasing circulating plasma levels of acyl ghrelin, while not effecting des-acyl ghrelin, and attenuates weight gain, but not food intake in mice (Barnett, Hwang, Taylor, Kirchner, Pfluger, Bernard, Lin, Bowers, Mukherjee, Song, Longo, Leahy, Hussain, Tschoep, Boeke, and Cole, 2010).

Therefore, the purpose of the present experiments was to test the necessity of ghrelin acylation via GOAT in food deprivation-induced increases in ingestive behaviors. This was accomplished by giving the GOAT inhibitor, GO-CoA-Tat, during food deprivation to inhibit ghrelin acylation, and then to measure food foraging, intake, and hoarding during refeeding.

Materials and Methods

Animals and Housing

Male Siberian hamsters (*Phodopus sungorus*) were used that were bred, born, and raised in our breeding colony that has been previously described (Bowers, Festuccia, Song, Shi, Migliorini, and Bartness, 2004). In brief, breeding pairs were housed in a 16L:8D photoperiod (light offset: 1800) with offspring weaned at 18 days of age and group housed by sex in polypropylene cages (456 × 234 × 200 mm) containing corn cob bedding (Bed-O-Cobs, The Andersons Inc., Maumee, OH) and two cotton Nestlets (Anacare, Belmore, NY) until selection for experimental use. Animals were given ad libitum access to rodent chow (Laboratory Rodent Diet 5001, Purina, St. Louis, MO) and tap water, unless otherwise noted. Room temperature was maintained at 21 ± 2 °C. All procedures were approved by the Georgia State University Institutional Animal Care and Use Committee and were in accordance with Public Health Service and United States Department of Agriculture guidelines.

Circulating Ghrelin Concentration

Thirty-six male Siberian hamsters were selected from our breeding colony at ~2.5 months of age and singly housed in an Optimice system (Animal Care Systems, Inc., Centennial, CO) with corncob bedding and one cotton nestlet. The animals were allowed to acclimate to the new housing regime for two weeks, recording body weight and food intake weekly. After

the two weeks of acclimation, baseline food intake and body weight were recorded every other day for one week. The animals were then separated into 2 treatment groups: a) GO-CoA-Tat (11 $\mu\text{mol/kg}$; $n=18$) and b) Saline ($n=18$) balanced for percent body mass change since single housing, and absolute body mass. These two groups were further separated into one of three blood collection times: 6-, 12-, and 24 h post-injection, resulting in 6 groups ($n = 6$). Injections were delivered intraperitoneally (i.p.) at ~1 h before light offset. GO-CoA-Tat was provided by Dr. Philip A. Cole and prepared as previously described using solid phase synthesis with introduction of the CoA by thiol displacement of the corresponding bromo-octanamide intermediate (Barnett *et al.*, 2010). Final GO-CoA-Tat compound was purified by reversed phase HPLC and the correct structure confirmed by mass spectrometry. The animals had free access to food and water during the interval before blood sampling and food intake and body weight were recorded at the time of blood sampling. Acyl ghrelin and des-acyl ghrelin were assayed with the blood collected differentially dependent upon the hormone (see directly below). Intraorbital blood was taken and circulating acyl ghrelin and des-acyl ghrelin concentrations were assessed using ELISA (ALPCO Immunoassays, Salem, NH) according to manufacturer's instructions and previous published methods (Barnett *et al.*, 2010). In brief, ~500 μl blood was collected via the retro-orbital sinus using heparinized Natelson collecting tubes. For the acyl ghrelin assay (A05117) 300 μl of the blood was put into a pre-chilled BD microtainers containing EDTA (Franklin Lakes, NJ), inverted 10 times, and placed on ice until all samples were obtained. The blood was then transferred to pre-chilled microcentrifuge tubes containing 300 μl of blood transfer buffer (1.2 % 10 N NaOH 2 mM p-hydroxymercuribenzoic acid, 500 mM NaCl, and 25 mM EDTA in deionized water) and then spun at 5,000 rpm at 4 °C for 10 min. Plasma was then transferred to pre-chilled microcentrifuge tubes and immediately acidified using 1 N HCl (1 μl HCl/10 μl plasma) and spun at 5,000 rpm at 4 °C for 5 min. The acidified plasma was transferred to a microcentrifuge tube and stored at -20 °C until assayed for acyl ghrelin. For des-acyl ghrelin, 200 μl of the remaining blood was put into a pre-chilled BD microtainers containing EDTA (Franklin Lakes, NJ), inverted 10 times, placed on ice until all samples were obtained, and then spun at 3,500 rpm at 4 °C for 10 min. The samples were then transferred to microcentrifuge tubes and stored at -20 °C until assayed for des-acyl ghrelin (A05118). Samples were diluted as necessary to be read in the midrange of the standard curve.

Food Foraging, Intake, and Hoarding

Forty male Siberian hamsters were selected from our breeding colony and transferred to a separate room 16L:8D (light offset: 1400) where they were singly housed in polypropylene cages for two weeks. Animals had *ad libitum* test diet (75 mg pellets: Dustless Precision Pellets, Purified 75 mg pellets; Bio-Serv, Frenchtown, NJ), Alpha-Dri bedding (Specialty Papers, Kalamazoo, MI), and one cotton nestlet. The animals acclimated to the new light offset and diet for two weeks, body mass was recorded weekly. At the conclusion of the two weeks the animals were transferred into the simulated-natural foraging/hoarding apparatus, previously described (Day and Bartness, 2001). Briefly, two cages are connected via polyvinyl chloride tubing (38.1 mm inner diameter and ~1.52 m in length) that requires vertical climbs/descents and horizontal runs to move between the bottom and top cage. The opaque bottom cage (290 × 180 × 130 mm), "home cage", contained alpha-dri bedding and one cotton nestlet and was covered with an aluminum pan to simulate the darkness of the burrow. The clear top cage (456 × 234 × 200 mm), "foraging cage", contained a water bottle, a running wheel, and food source. The running wheel (524 mm) was connected to a computer based hardware/software interface (Med Associates, Georgia, VT) that transmitted wheel rotations via a magnet switch system that would subsequently dispense a food pellet after 10 wheel rotations were completed. For the first 3 d in the foraging/hoarding apparatus each animal was given 300 pellets and able to earn food via wheel running (foraging), the animals were then allowed to acclimate to earning all of their food (10 rotations/pellet) for 2

wk. Each day the animals were in the foraging/hoarding apparatus wheel rotations were recorded and used to determine the number of food pellets foraged (wheel rotations/10). Food hoarded was defined as the food found in the hamster's cheek pouches and home cage and surplus food was defined as the food found in the top cage (neither eaten nor hoarded). Food intake was defined as pellets foraged – (surplus food + food hoarded) The behavioral data from the final week of the acclimation period was used as the baseline by which foraging treatment groups were determined along with body mass and percent body mass change over the week. An electronic scale used to weigh the food pellets was set to “parts” measurement, resulting in one 75 mg food pellet = 1 with fractions of pellets computed by the scale. After data collection the surplus and hoarded pellets were discarded.

The animals were then assigned to one of three foraging treatments: 1) food delivery contingent upon completing 10 wheel revolutions (10REV), 2) non-wheel running contingent food available (300 pellets) with an operational running wheel (free wheel group; FW), and 3) non-wheel running contingent food available (300 pellets) with a locked running wheel (blocked wheel group; BW).

After 1 wk in their respective foraging treatments, all animals were food deprived for 48 h, beginning at light offset (1400). Each animal received an i.p. injection of either GO-CoA-Tat (11 μ mol/kg) or saline every 6 h during the 48 h of food deprivation because GO-CoA-Tat significantly inhibits acylated ghrelin production for only 6 h (Barnett *et al.*, 2010), resulting in 9 injections. After the final injection, which coincided with light offset, animals had food returned and wheel rotations (food foraged), food intake, and food hoarding were recorded at 1-, 2-, 4-, 24 h post-final injection and the days subsequent until all behaviors returned to pre-FD baseline values.

Statistical Analysis

Circulating concentrations of acyl- and des-acyl ghrelin were analyzed using a t-test within each time point. Behavioral data was analyzed using two-way ANOVA (foraging treatment X injection treatment) within time points, because of unequal time interval durations no comparisons were made between time points. Post hoc tests for behavioral data were conducted with Duncan's New Multiple Range test. All analyses done using NCSS (version 2007, Kaysville, UT) and exact probabilities and test values were omitted for simplicity and clarity of presentation. Statistical significance was considered if $P < 0.05$.

Results

Circulating Ghrelin Concentration

The circulating plasma concentration of des-acyl ghrelin was not different at any of the time points examined in the GOAT inhibitor-treated animals compared to the saline-treated controls (Fig. 1A). Animals treated with the GOAT inhibitor had significantly reduced plasma circulating concentrations of acyl ghrelin 6 h after treatment when compared with saline treatment, but not at 12- and 24 h post-injection as expected (Fig. 1B).

Food Foraging, Intake, and Hoarding

Food foraging was inhibited for 1 h post-refeeding in the GOAT inhibitor-treated animals compared with saline-treated animals, but at no other time point (Fig. 2B) and surprisingly increased wheel running 3 d post-refeeding. It is important to note that this inhibition is not due to sickness or general malaise in the animals as can be seen in the lack of an activity deficit in the FW group (Fig. 2A) and observation of the animals (*e.g.*, maintenance of their coat). Food intake was inhibited compared with the saline-treated animals at 0–1 and 2–4 h post-refeeding in the 10REV foraging treatment (Fig. 3C) and an apparent delayed

compensatory increase in food intake occurred for these hamsters on Day 4. Food intake was generally not affected in the BW group except for an apparent anomalous increase on Day 4 (Fig. 3A). Food intake was not affected at any time point in the FW (Fig. 3B). Food hoarding was inhibited at 0–1 h and on *Day 2* and *Day 3* post-refeeding when compared with saline-treated animals in the 10REV (Fig. 4C) foraging group and was not affected at any time point in the BW (Fig. 4A) and FW (Fig. 4B) foraging groups.

Discussion

The present experiments tested the necessity of acyl ghrelin in the food deprivation-induced increases in appetitive and consummatory ingestive behaviors using the GOAT inhibitor, GO-CoA-Tat in Siberian hamsters housed in our simulated burrow system. We found that systemically administered GO-CoA-Tat given across the 48 h food deprivation period inhibits octanoylation of ghrelin by GOAT critical for its orexigenic functions as evidenced by the reduction in the circulating concentration of acyl ghrelin for at least 6 h, an effect similar to that reported for mice (Barnett *et al.*, 2010). This GOAT inhibition attenuated food foraging (0–1 h), food intake (0–1 and 2–4 h), and food hoarding (0–1 h, 2 d, and 3 d) in animals that were required to earn their food (10REV), with the inhibition of food hoarding being the most prolonged. To our knowledge, this is the first demonstration that GOAT blockade inhibits food intake and moreover food deprivation-induced increases in food hoarding and foraging in any species. Although we cannot completely rule out off-target effects of GO-CoA-Tat in contributing to changes in food intake, the inhibitor showed no obvious toxicity in mice after 30 d treatment (Barnett *et al.*, 2010). As noted above, these effects of GO-CoA-Tat only were seen in the 10REV foraging group, a restriction that is not unique to this study. For example, food deprivation-induced increases in food hoarding primarily were inhibited in the 10REV foraging groups after melanotan II [MTII (Keen-Rhinehart and Bartness, 2007a)], an agonist of the melanocortin 4-receptor, and 1229U91 (Keen-Rhinehart and Bartness, 2007b), a neuropeptide Y (NPY) Y1 receptor antagonist.

Food deprivation elicits many physiological changes in addition to increases in circulating acyl ghrelin concentrations, representing several seemingly redundant signals/mechanisms to increase ingestive behaviors/decrease energy expenditure [for review see: (Grill, 2006; Zheng, Lenard, Shin, and Berthoud, 2009)]. Due to these multiple signals, it is not surprising that the ingestive behaviors examined here were not completely inhibited at any single time period nor across the duration of the behavioral test. Furthermore, given the short (~6 h) effect of GO-CoA-Tat on acylated ghrelin production, the truncated behavioral effects also are not surprising. GO-CoA-Tat inhibited food hoarding on the 2nd and 3rd d post refeeding, a time when acyl ghrelin concentrations would no longer be affected by GO-CoA-Tat. This suggests that early inhibition of the normally increased endogenous ghrelin with food deprivation, albeit temporary, can trigger a sustained reduction in food hoarding. This result is somewhat ‘reversely analogous’ to the stimulation of food intake by centrally administered agouti-related protein (AgRP) in laboratory rats that lasts for several days, but only is transiently inhibited by MTII administration 24 h *after* AgRP treatment (Hagan, Rushing, Pritchard, Schwartz, Strack, Van Der Ploeg, Woods, and Seeley, 2000). This prolonged inhibition of food hoarding by GO-CoA-Tat and the prolonged stimulation of food intake by AgRP (Hagan, Benoit, Rushing, Pritchard, Woods, and Seeley, 2001; Hagan *et al.*, 2000; Wirth and Giraudo, 2000) as well as the prolonged (~5–7 d) increases in food hoarding by AgRP (Day and Bartness, 2004) and systemic ghrelin injection (Keen-Rhinehart *et al.*, 2005; Keen-Rhinehart *et al.*, 2007a; Keen-Rhinehart *et al.*, 2007b), clearly demonstrate our lack of understanding of sustained changes in ingestive behaviors when the immediate effects of stimuli inhibiting or stimulating appetitive and/or consummatory ingestive behaviors are no longer present.

In the present experiments we found that GO-CoA-Tat attenuated food foraging (0–1 h), food intake (0–1 and 2–4 h), and food hoarding (0–1 h and 2 and 3 d) post-refeeding compared with saline-treated animals. We previously found that antagonism of receptors for NPY and AgRP, proven downstream ghrelin targets that increase food intake in laboratory rats and mice [for review see: (Briggs and Andrews, 2011)], inhibited food deprivation-induced increases in foraging and hoarding in Siberian hamsters (Dailey and Bartness, 2009; Keen-Rhinehart *et al.*, 2007a; Keen-Rhinehart *et al.*, 2007b). The present effects are consistent with the previously hypothesized scenario (Bartness *et al.*, 2011) whereby food deprivation triggers increases in acylated circulating ghrelin concentrations that, in turn, stimulate GHSRs in several brain and peripheral sites including engaging the GHSRs found on Arc NPY/AgRP neurons increasing their synthesis and likely release at projections in several sites including the hypothalamic paraventricular nucleus, perifornical area, as well as perhaps co-localized γ -aminobutyric acid (GABA) in the parabrachial nucleus [PBN; (Wu, Boyle, and Palmiter, 2009)]. In terms of the latter, we find that the GABA-a receptor agonist, muscimol nanoinjected into the PBN mimics much of the increased duration and degree of food hoarding by food deprivation, ghrelin or 3V AgRP (Teubner, B. J. W. and Bartness, T. J., unpublished observations).

GHSRs also are located in the ventral tegmental area [VTA; (Guan, Yu, Palyha, McKee, Feighner, Sirinathsinghji, Smith, Van Der Ploeg, and Howard, 1997)] which is part of the mesolimbic dopamine circuitry that is thought to be intimately involved in the reinforcing aspects of food intake [for review see: (Narayanan, Guarnieri, and DiLeone, 2010)]. Indeed, GHSRs are located on dopaminergic neurons in the VTA (Zigman, Jones, Lee, Saper, and Elmquist, 2006) and ghrelin administration causes dopamine release in the nucleus accumbens (Jerlhag, Egecioglu, Dickson, Douhan, Svensson, and Engel, 2007). Moreover, ghrelin administration into the VTA increases food intake (Naleid, Grace, Cummings, and Levine, 2005) and GHSR antagonism preferentially attenuates the intake of palatable food (Egecioglu, Jerlhag, Salome, Skibicka, Haage, Bohlooly, Andersson, Bjursell, Perrissoud, Engel, and Dickson, 2010). These data collectively suggest that ghrelin is involved in the reinforcing effects of food [for review: (Kang *et al.*, 2011; Skibicka and Dickson, 2011)]. The prevention of increases in food intake by peripheral ghrelin administration when a GHSR antagonist (BIM28163) is administered into the ventral tegmental area in laboratory rats (Abizaid, Liu, Andrews, Shanabrough, Borok, Elsworth, Roth, Sleeman, Picciotto, Tschop, Gao, and Horvath, 2006) suggests that activation of GHSRs in the VTA alone may be necessary for peripheral ghrelin-induced increases in food intake despite the focus on activation of these receptors in the Arc [*e.g.*, (Currie, Mirza, Fuld, Park, and Vasselli, 2005; Tamura, Kamegai, Shimizu, Ishii, Sugihara, and Oikawa, 2002)]. These latter data create a seemingly complex interrelation between the Arc and VTA as well as other sites implicated in differing aspects controlling ingestive behavior [*e.g.*, the amygdala and anxiety when food is absent (Alvarez-Crespo, Skibicka, Farkas, Molnar, Egecioglu, Hrabovszky, Liposits, and Dickson, 2012)]. A possible underlying basis connecting the Arc, VTA and perhaps the amygdala, is that ghrelin is involved in foraging for food, as we previously found with peripheral ghrelin administration to Siberian hamsters (Keen-Rhinehart *et al.*, 2005; Keen-Rhinehart *et al.*, 2007a; Keen-Rhinehart *et al.*, 2007b) and as has been recently suggested for the VTA in an operant model of food procurement in laboratory rats (Skibicka, Hansson, Alvarez-Crespo, Friberg, and Dickson, 2011). Therefore, experiments manipulating the ghrelin/dopamine system within the mesolimbic dopamine pathway could prove fruitful in elucidating the mechanism behind food the appetitive ingestive behaviors of food foraging and hoarding.

In summary, preventing the acylation of ghrelin using the GOAT inhibitor, GO-CoA-Tat, attenuates the food deprivation-induced increases in both appetitive (foraging and hoarding) and consummatory (food intake) ingestive behaviors. The incomplete inhibition of ingestive

behavior suggests that there are multiple mechanisms that help ensure the return to energy homeostasis continues to progress during metabolic challenges, here extended food deprivation. The presence of multiple, seemingly redundant, mechanisms some of which are undefined at this time, further underscores the importance of energy acquisition. These multiple mechanisms present a problem to the prevention and reversal of obesity, where a complete intervention will necessitate multiple points of treatment.

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Highlights

- Fasting and ghrelin injection increase food foraging, intake & especially hoarding
- Ghrelin acylation by ghrelin *O*-acyltransferase (GOAT) activates ghrelin
- GOAT inhibition decreased fasting-induced food foraging, intake and hoarding
- Octanoylated ghrelin affects fasting-induced increases these ingestive behaviors

Figure 1.

Mean circulating plasma concentrations $\pm$ S.E. at 6, 12, and 24 h after a single intraperitoneal injection of GOAT inhibitor, GO-CoA-Tat (11 μ mol/kg), or saline at light offset of (A) des-acyl ghrelin (pg/ml) and (B) acyl ghrelin (pg/ml). * $P < 0.05$ vs. saline treat animals within time point.

Figure 2.

Mean number of wheel rotations $\pm$ S.E. at 0–1, 1–2, 2–4, and 4–24 h and 2, 3, and 4 d post-refeeding after 48 h food deprivation in animals that received GO-CoA-Tat (11 μ mol/kg) or saline i.p. in (A) free wheel/free food or (B) 10 wheel rotations/pellet foraging treatments. * $P < 0.05$ vs. saline treated animals within time point.

Figure 3.

Mean number of food pellets eaten $\pm$ S.E. at 0–1, 1–2, 2–4, and 4–24 h and 2, 3, and 4 d post-refeeding after 48 h food deprivation in animals that received GO-CoA-Tat (11 μ mol/kg) or saline i.p. in (A) blocked wheel/free food, (B) free wheel/free food or (C) 10 wheel rotations/pellet foraging treatments. * $P < 0.05$ vs. saline treated animals within time point.

Figure 4.

Mean number of food pellets hoarded $\pm$ S.E. at 0–1, 1–, 2–4, and 4–24 h and 2, 3, and 4 d post-refeeding after 48 h food deprivation in animals that received GO-CoA-Tat (11 μ mol/kg) or saline i.p. in (A) blocked wheel/free food, (B) free wheel/free food or (C) 10 wheel rotations/pellet foraging treatments. * $P < 0.05$ vs. saline treated animals within time point.