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MTII attenuates ghrelin- and food deprivation-induced increases in food hoarding and food intake

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Abstract

Food deprivation triggers a constellation of physiological and behavioral changes including increases in peripherally-produced ghrelin and centrally-produced agouti-related protein (AgRP). Upon refeeding, food intake is increased in most species, however hamsters primarily increase food hoarding. Food deprivation-induced increases in food hoarding by Siberian hamsters are mimicked by peripheral ghrelin and central AgRP injections. Because food deprivation stimulates ghrelin as well as AgRP synthesis/release, food deprivation-induced increases in hoarding may be mediated by melanocortin 3 or 4 receptor (MC3/4-R) antagonism via AgRP, the MC3/4-R inverse agonist. Therefore, we asked: Can a MC3/4-R agonist block food deprivation- or ghrelin-induced increases in foraging, food hoarding and food intake? This was accomplished by injecting melanotan II (MTII), a synthetic MC3/4-R agonist, into the 3rd ventricle in food deprived, fed or peripheral ghrelin injected hamsters and housed in a running wheel-based food delivery foraging system. Three foraging conditions were used: a) no running wheel access, non-contingent food, b) running wheel access, non-contingent food or c) a foraging requirement for food (10 revolutions/pellet). Food deprivation was a more potent stimulator of foraging and hoarding than ghrelin. Concurrent injections of MTII completely blocked food deprivation- and ghrelin-induced increases in food intake and attenuated, but did not always completely block, food deprivation- and ghrelin-induced increases in food hoarding. Collectively, these data suggest that the MC3/4-R are involved in ghrelin- and food deprivation-induced increases in food intake, but other neurochemical systems, such as previously demonstrated with neuropeptide Y, also are involved in increases in food hoarding as well as foraging.

Keywords: Fasting; Foraging; Melanotan II; Hamster; Siberian hamster; Intracerebroventricular

Introduction

Determining the physiological factors that regulate ingestive behavior is critical to understanding the etiology of obesity. Ingestive behavior, as with other goal-oriented behaviors, 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 (Craig, 1918). The consummatory aspects of ingestive behavior have received most of the 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 these behaviors, which is surprising, given its pervasive nature across animal taxa (for review see: Illius et al., 2002). Food hoarding, the storage of food for later ingestion, has widespread expression among animal species (for review see: Vander Wall, 1990), but the mechanisms underlying this appetitive ingestive behavior have received little attention compared with food intake (for review see: Bartness and Day, 2003). Perhaps the lack of attention to the appetitive phase of ingestive behavior is due to the difficulty in conducting field studies of hoarding or the problem of creating a laboratory-based analog of this behavior.

Siberian hamsters (*Phodopus sungorus*) and other hamster species (for review see: Bartness and Demas, 2004) primarily increase foraging (Day and Bartness, 2003; Bartness and Day, 2003) and food hoarding (Bartness and Clein, 1994; Wood and Bartness, 1996; Bartness, 1997) in response to energetic

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challenges, rather than food intake as with laboratory rats and mice (for review see: Bartness and Day, 2003). Siberian hamsters, and other animals that have the capacity to transport significant amounts of food (for review see: Vander Wall, 1990), use food hoarding as a crucial part of their ingestive behavioral repertoire in response to many naturally occurring energetic challenges (*e.g.*, pregnancy, lactation (Bartness, 1997; Bartness and Day, 2003); for review see: Bartness and Day, 2003).

Another naturally occurring energetic challenge is decreased food availability and in its extreme, food deprivation, a condition that triggers changes in a plethora of peripheral metabolism alterations, peripheral signaling peptides and central neurochemicals (for reviews see: Newsholme and Leech, 1983; Konturek et al., 2004). Upon refeeding, there are marked increases in appetitive ingestive behaviors in Siberian hamsters, with relatively minor changes in food intake (Bartness and Clein, 1994; Wood and Bartness, 1996; Bartness, 1997; Day and Bartness, 2003). The exact mechanisms underlying these food deprivation-induced increases in appetitive ingestive behaviors are unknown, but there are increases in peripheral and central peptide synthesis/release implicated in the stimulation of food intake that are associated with food deprivation in these and other animals. For example, food deprivation triggers increases in circulating concentrations of the largely stomach-derived peptide ghrelin in Siberian hamsters (Keen-Rhinehart and Bartness, 2005), as it does in laboratory rats (*e.g.*, Tschop et al., 2000; Sun et al., 2003). In addition, peripherally administered ghrelin that creates 24–48 h food deprivation-like plasma active ghrelin concentrations markedly stimulates food hoarding and, to a lesser degree, food intake in Siberian hamsters (Keen-Rhinehart and Bartness, 2005). Food deprivation also increases arcuate nucleus gene expression of the orexigenic peptides neuropeptide Y (NPY) and agouti-related peptide (AgRP) in Siberian hamsters (Mercer et al., 1995; Mercer et al., 2000), as it does in laboratory rats and mice (*e.g.*, Brady et al., 1990; Kim et al., 1998; Mizuno et al., 1999; Mizuno and Mobbs, 1999). When AgRP (Day and Bartness, 2004), NPY (Day et al., 2005) or a NPY Y1 receptor agonist ([Pro34]NPY; Day et al., 2005) are administered centrally to Siberian hamsters, food hoarding strikingly increases whereas food intake minimally increases or does not increase. Thus, food deprivation increases circulating ghrelin concentrations (Tschop et al., 2000; Sun et al., 2003; Keen-Rhinehart and Bartness, 2005) that, in turn, stimulate NPY/AgRP-producing arcuate neurons (*e.g.*, Guan et al., 1997, 2003; Kohno et al., 2003; Seoane et al., 2003) and presumably NPY/AgRP synthesis/release (Wren et al., 2002) finally acting on melanocortin 3 and 4 receptors (MC3/4-R) in the hypothalamic paraventricular nucleus and other areas. Therefore, antagonism of these downstream MC3/4-Rs would appear to at least partly underlie food deprivation- and ghrelin-induced increases in appetitive ingestive behaviors. Therefore, we asked: Can an MC3/4-R agonist (melanotan II [MTII]) block food deprivation- or ghrelin-induced increases in foraging and food hoarding? This was accomplished by attempting to block food deprivation- and peripheral ghrelin-induced increases in foraging and food hoarding by injecting MTII into the third ventricle of food deprived, fed or ghrelin-injected hamsters housed in a running wheel-based food delivery foraging system that is coupled with simulated burrow-housing.

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 months old and weighing 35–43 g were obtained from our breeding colony. The lineage of this colony has been described recently (Bowers et al., 2004). Hamsters were group-housed and raised in a long-day photoperiod (16:8 light:dark; lights on at 0200 h) from birth. Room temperature was maintained at 21±2.0 °C.

Hamsters were acclimated for 2 weeks in specially designed hoarding apparatuses as previously described (Day and Bartness, 2001; Day et al., 2005) that would serve as their housing for the duration of the experiment. More specifically, 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 diet (75 mg pellets: Purified Rodent Diet; Research Diets, New Brunswick, NJ) and tap water were available *ad libitum*. A running wheel (524 mm circumference) and pellet dispenser were attached to the food cage (top). Wheel revolutions were counted using a magnetic detection system and monitored by a computer-based hardware–software program (Med Associates, Lancaster, NH). Hamsters were first trained in these apparatuses (Day et al., 2005; Keen-Rhinehart and Bartness, 2005) and then received a third ventricular cannula both previously described (Day and Bartness, 2004; Day et al., 2005) and described in brief below.

Foraging training regimen

We used a wheel-running training regimen that eases the hamsters into their foraging efforts without large changes in body mass or food intake (Day and Bartness, 2001). Specifically, hamsters were given free access to the food pellets for 2 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 third 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 revolutions and pellets earned (foraging) were measured daily. At the end of this acclimation period (7 days total), all animals were removed from the foraging apparatuses and temporarily housed in shoebox cages where the same food pellets were available *ad libitum* with no foraging requirements. Guide cannulae were then stereotaxically implanted in these hamsters (see below for details). Following a 1-week post surgical recovery period, all hamsters were returned to the hoarding/foraging apparatus and retrained to the following schedule: 2 days for adaptation with free access to food pellets followed by 5 days at 10 revolutions/pellet. Hamsters remained in the hoarding/foraging apparatus for the remainder of the experiment.

Cannula implantation

Cannulae were stereotaxically implanted into the third ventricle as described previously (Day and Bartness, 2004). Briefly, the animals were anesthetized with isoflurane and the fur at the top of the head was removed to expose the area to be incised. A hole was trephined at the intersection of bregma and the midsaggital sinus and the guide cannula (26 gauge stainless steel; Plastics One, Roanoke, VA) was lowered using the following stereotaxic coordinates (level skull, anterior–posterior from bregma 0, medial–lateral from midsaggital sinus 0, and dorsal–ventral from the top of the skull −5.0 mm) targeted for placement just above the third ventricle. The guide cannula was secured to the skull using cyanoacrylate ester gel, 3/16 mm jeweler's screws and dental acrylic. A removable obturator sealed the opening in the guide cannula throughout the

experiment except when it was removed for the injections. Hamsters received 0.2 mg/kg buprenorphine at 12 and 24 h post-surgery to minimize discomfort, were given fresh apple bits to encourage food/fluid intake and subsequently were allowed 1 week to recover fully in the shoebox cage housing before being returned to their simulated burrow housing.

Cannulae verification

Following the last test, an injection of 0.4 μl of bromophenol blue dye was given to confirm placement of the cannula in the third ventricle. The animals were killed with an overdose of pentobarbital sodium (75 mg/kg), their brains removed and then postfixed in 10% paraformaldehyde for a minimum of two d. Each brain was sliced manually for cannula verification. Cannulae were considered to be located in the third ventricle if the dye was visible in any part of this ventricle. Only the data from animals with confirmed third ventricle cannulae placements were included in the analyses. There was no incidence of cannula loss during the study.

Intracerebroventricular injection protocol

The inner cannula (33 gauge stainless steel, Plastics One, Roanoke, VA) extended 5.5 mm below the top of the skull and all hamsters were injected with a 0.4 μl volume. All injections were given at the beginning of the dark phase of the photoperiod. Animals were lightly restrained by hand during the 30 s injection and the injection needle remained in place ~30 s before withdrawal to minimize injectate reflux, according to our previous procedure (Day and Bartness, 2004; Day et al., 2005; Keen-Rhinehart and Bartness, 2007).

Experimental design

At the end of the 7-day retraining period, the hamsters were separated into three groups ($n=8$/group) matched for their current body mass and average hoard size across these last 3 days of training while at 10 revolutions/pellet. The three groups consisted of: 10 revolutions/pellet foraging requirement (10 Revolutions/pellet group), no foraging requirement with an active running wheel (Free Wheel; exercise control group) or no foraging requirement with a blocked wheel (Blocked Wheel; sedentary control group) where each of the last two groups had food available non-contingently. For Experiment 1, each group received all drug combinations given in a counterbalanced schedule to control for possible order effects of peptide administration (see below for details). For Experiment 2, each group was observed during a baseline *ad libitum*, saline-treated period that was followed by 48 h of food deprivation, and behavioral measurements were then performed under either saline or MTII treatment and compared with the baseline measurements (see below for details).

Measurement of foraging, food hoarding and food intake

Foraging (pellets earned) was defined as the number of pellets delivered upon completion of the requisite 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. For the 10 Revolutions/pellet groups, 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 − pellets left in the top cage − hoarded pellets = food intake. An electronic balance used to weigh the food pellets was set to 'parts' measurement rather then obtaining fractions of a pellet in mg; thus one 75 mg food pellet = 1.

Experiment 1: Does MTII inhibit ghrelin-induced stimulation of appetitive and consummatory ingestive behaviors in Siberian hamsters?

A within-subjects design was chosen to minimize variability; therefore, all animals received all of the following possible injection combinations: ip ghrelin (30 μg/kg, Bachem Biosciences, King of Prussia, PA) + MTII (2.5 nmol, Phoenix Pharmaceuticals, Belmont, CA), ip saline + icv MTII (2.5 nmol), ip ghrelin (30 μg/kg) + icv saline, ip saline + icv saline. This dose of ghrelin was chosen because it results in plasma active ghrelin concentrations within the physiological

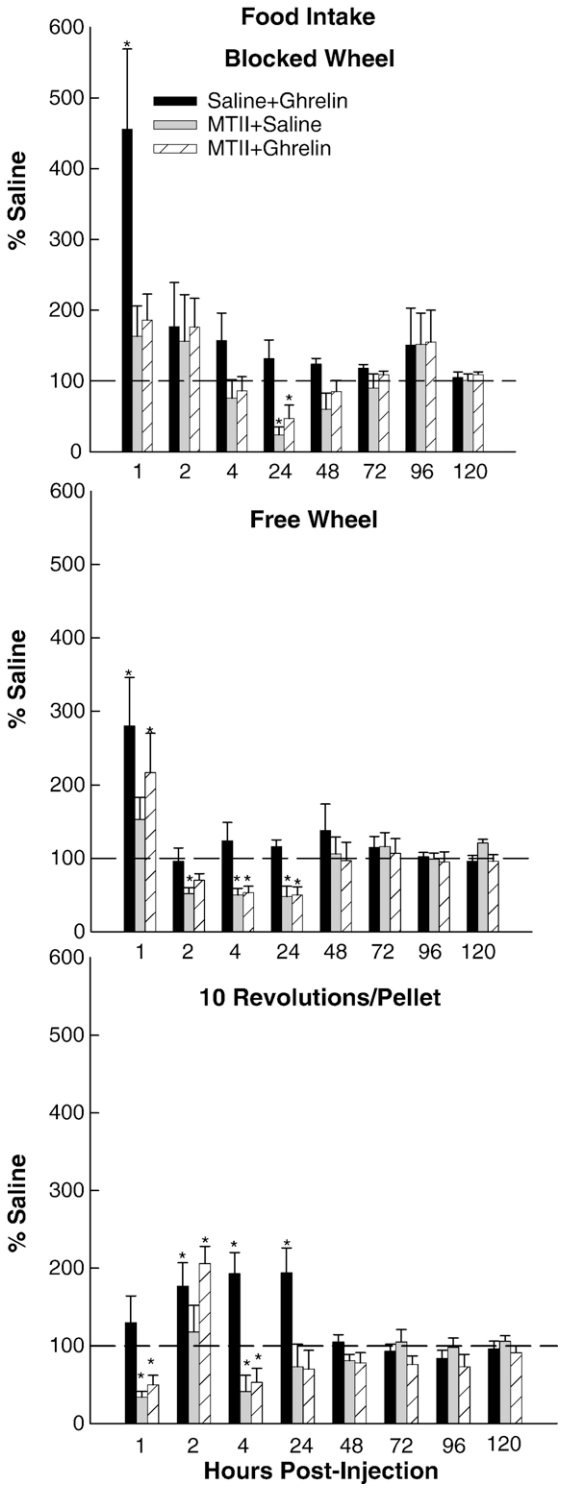

Fig. 1. Mean ± S.E.M. of food intake as a percentage of the intracerebroventricularly (icv) and intraperitoneally (ip) saline-injected controls (dashed reference line) for the effects of ip ghrelin treatment with icv saline (black bars), ip saline treatment with icv melanotan II (MTII; gray bars) and ip ghrelin treatment with icv MTII (striped bars) on hamsters without a foraging requirement and a stationary wheel (Blocked Wheel), hamsters with no foraging requirement and a freely moving wheel (Free Wheel) and hamsters with a foraging requirement (10 Revolutions/pellet). * = $P<0.05$ compared to the saline control condition.

range of food deprivation for 12–48 h in Siberian hamsters (Keen-Rhinehart and Bartness, 2005). Because there were no carry-over effects of ghrelin at this dose for any behavior beyond 7 days (Keen-Rhinehart and Bartness, 2005) a washout period of this length occurred between the counterbalanced injections. The dose of MTII was chosen based on 3rd ventricular injections of this melanocortin receptor agonist that inhibit food intake in this species (Schuhler et al., 2003; Schuhler et al., 2004) as well as a pilot study testing for a MTII dose that inhibited both food intake and food hoarding — the 2.5 nmol dose fulfilled this criterion. Note that because there were no significant changes in body mass for any group after any of the injections, the dose of ghrelin was kept constant.

Experiment 2: Does the MC3/4-R agonist, MTII, inhibit food deprivation-induced stimulation of appetitive and consummatory ingestive behaviors in Siberian hamsters?

Two weeks after the last injection from Experiment 1, animals were divided into two groups, re-balanced for body mass (even though there was no significant changes in body mass throughout the first experiment [data not shown]) as well as for hoarding and were then food deprived for 48 h beginning at lights-out. Half of the animals received icv MTII (2.5 nmol) and the other half received icv saline. In our previous studies of food hoarding, we have used food deprivation ranging from 12 to 56 h (IACUC approved) with the latter length appearing somewhat lengthy or 'non-physiological' at first blush. In the utopian conditions of the laboratory, however, Siberian hamsters are almost 50% body fat compared to as low as ~25% in nature (Weiner, 1987); therefore, short fasts in the laboratory of 12–32 h are minimally energetically challenging in these animals and thus stimulation of food hoarding is minimal (Clein and Bartness, unpublished results). Therefore, we selected 48 h food deprivation to trigger the behavior nearly maximally. It also seems reasonable to envision these fast lengths as on a physiological continuum with the inter-meal intervals occurring naturally of much shorter lengths in hamsters (~4 h; Bartness et al., 1986).

Statistical analyses

For all measures of food intake, foraging and food hoarding in Experiments 1 and 2, if the saline treated animals did not eat, forage (earn pellets) or hoard at a time interval (*i.e.*, a value of '0'), the minimum value (1 pellet) was assigned to avoid a zero in the denominator for calculation of the percent changes from saline for these measures. In Experiment 1, each animal was given saline+saline, ghrelin+saline, MTII+saline, and ghrelin+MTII. The percent saline value was calculated by dividing each animal's behavioral response to each of the drug conditions by that of the saline+saline control condition multiplied by 100; thus, this yields data for each animal expressed as a percentage of their own saline values. All the individual percent saline values were then averaged to generate a mean across animals for each foraging condition with associated standard errors. In Experiment 2, food intake, foraging and food hoarding were monitored during an *ad libitum* -feeding, saline injection baseline period and then each animal was food deprived followed by icv injection of either saline or MTII. As in Experiment 1, the percent saline value was calculated by dividing each animal's behavioral response to either food deprivation+saline or food deprivation +MTII by each animal's *ad libitum* saline baseline value multiplied by 100; thus, this yields data for each animal expressed as a percentage of their own saline values. Once again, all the individual percent saline values were then averaged to generate a mean across animals for each foraging condition with associated standard errors. The data for both experiments are graphed as the mean percent of saline±S.E.M. for food intake, food hoarding, foraging (10 Revolutions/pellet group) and wheel running (Free Wheel group). Because the data are analyzed for each time interval in order to identify intervals within which any experimental effects occured, rather than cumulatively, no statistical comparisons were made across time (*i.e.*, there was no Time factor analysis). In addition, comparisons among time intervals would be inappropriate, as the intervals are of differing durations. Therefore, the data from one time interval only are compared with data within that time interval. Data were analyzed using a repeated measures two-way ANOVA (Experiment 1: Foraging group×Drug Combination (3×4) at each of the 8 time periods; Experiment 2: Foraging group×Drug treatment (3×2) at each of the 8 time periods. Bonferroni's post-hoc tests were used for individual pair-wise comparisons (NCSS v 2000,

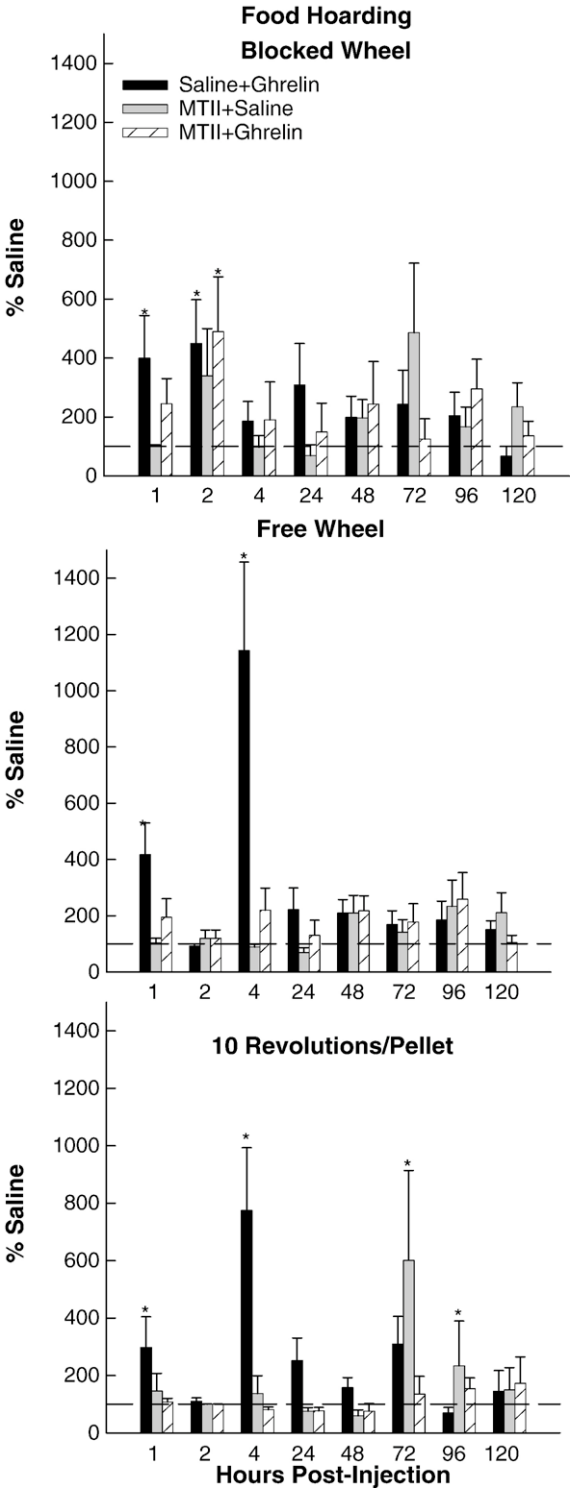

Fig. 2. Mean±S.E.M. of food hoarding as a percentage of the intracerebroventricularly (icv) and intraperitoneally (ip) saline-injected controls (dashed reference line) for the effects of ip ghrelin treatment with icv saline (black bars), ip saline treatment with icv melanotan II (MTII; gray bars) and ip ghrelin treatment with icv MTII (striped bars) on hamsters without a foraging requirement and a stationary wheel (Blocked Wheel), hamsters with no foraging requirement and a freely moving wheel (Free Wheel) and hamsters with a foraging requirement (10 Revolutions/pellet). *=$P<0.05$ compared to the saline control condition.

Kaysville, UT). 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 MTII inhibit ghrelin-induced stimulation of appetitive and consummatory ingestive behaviors in Siberian hamsters?

Wheel running

As seen previously (Keen-Rhinehart and Bartness, 2005), ghrelin did not stimulate wheel running activity when it was uncoupled from foraging (Free Wheel group) suggesting there was no non-specific stimulation or inhibition of locomotor activity by the peptide (data not shown).

Foraging

Ghrelin injections did not significantly stimulate foraging compared to saline (data not shown).

Food intake

Ghrelin significantly stimulated food intake (range: ~200–450%) at 0–1 h post-injection compared with saline for the Free Wheel and Blocked Wheel groups and at 1–2, 2–4, and 4–24 h for the 10 Revolutions/pellet group (Ps<0.05; Fig. 1). MTII completely blocked ghrelin-induced increased food intake at 0–1 h in the Blocked Wheel group, 2–4 and 4–24 h in the 10 Revolutions/pellet group (Ps<0.05; Fig. 1), but not at 1–2 h, and did not block food intake in the Free Wheel group at 0–1 h (Fig. 1). MTII alone and MTII+ghrelin treatment further decreased food intake compared with saline treatment at 4–24 h in the Blocked Wheel group, at 2–4 and 4–24 h in the Free Wheel group and at 2–4 h in the 10 Revolutions/pellet group (Ps<0.05; Fig. 1). In addition, MTII alone, but not MTII+ghrelin decreased food intake compared with saline at 1–2 h in the Free Wheel group and at 0–1 h in the 10 Revolutions/pellet group.

Food hoarding

Ghrelin significantly increased hoarding (range: ~300–400%) at 0–1 h post-injection for all groups compared with saline injections (Ps<0.05; Fig. 2). Ghrelin also increased hoarding at 1–2 h in the Blocked Wheel group (400%) and at 2–4 h in the Free Wheel group (1100%; Ps<0.05; Fig. 2). In addition, ghrelin significantly increased hoarding in the 10 Revolutions/pellet group at 2–4, 4–24 and 48–72 h post-injection compared with saline (range: ~200–800%, Ps<0.05; Fig. 2).

MTII blocked the ghrelin-induced increased hoarding at 0–1 h post injection in the Blocked Wheel group ($P<0.05$; Fig. 2), but not at 1–2 h. MTII completely blocked ghrelin-induced increased hoarding in the Free Wheel group at 0–1 and 2–4 h (Ps<0.05; Fig. 2). MTII also completely blocked the ghrelin-induced increased food hoarding in the 10 Revolutions group at all but 48–72 h post-injection (Ps<0.05; Fig. 2) at which time MTII treatment alone unexpectedly stimulated food hoarding compared with saline ($P<0.05$; Fig. 2).

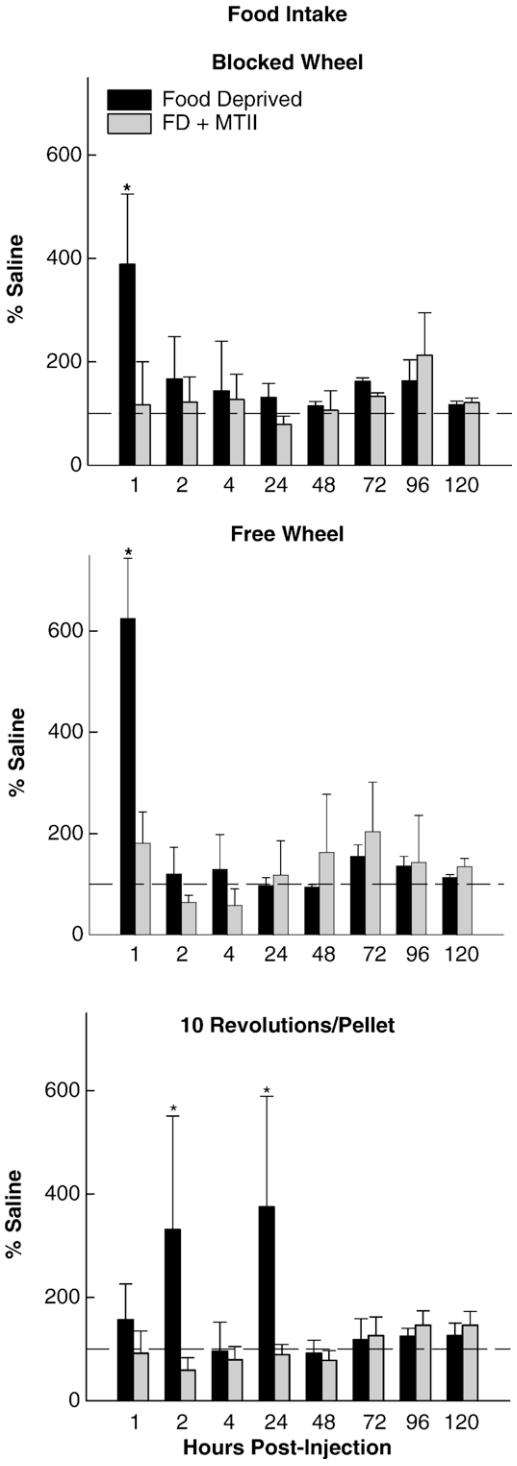

Fig. 3. Mean±S.E.M. food intake as a percentage of the saline-injected *ad libitum* fed control condition for the effects of food deprivation with intracerebroventricular (icv) saline injection (black bars) and food deprivation with icv melanotan II (MTII; gray bars) on hamsters without a foraging requirement and a stationary wheel (Blocked Wheel), hamsters with no foraging requirement and a freely moving wheel (Free Wheel) and hamsters with a foraging requirement (10 Revolutions/pellet). *=$P<0.05$ compared to saline injected *ad libitum* control condition.

Experiment 2: Does the MC3/4-R agonist, MTII, inhibit food deprivation-induced stimulation of appetitive and consummatory ingestive behaviors in Siberian hamsters?

Wheel running

Neither food deprivation nor MTII + food deprivation had any effect on wheel running (Free Wheel group; data not shown) suggesting there was no non-specific effects on locomotor activity.

Foraging

Food deprivation did not affect foraging, nor did MTII injections or their interactions (data not shown).

Food intake

Food deprivation increased food intake at 0–1 h in the Blocked Wheel and Free Wheel groups and at 1–2 and 4–24 h post injection in the foraging hamsters (10 Revolutions group; range: ~300–600%; Ps < 0.05; Fig. 3). These food deprivation-induced increases in food intake were smaller than the increases in food hoarding (see below). MTII blocked the food deprivation-induced increases in food intake by all groups at all time points (Fig. 3).

Food hoarding

Food deprivation stimulated food hoarding in all groups compared with *ad libitum* -fed hamsters (range: ~1000–3200%; Fig. 4). Specifically, the smallest significant increase in food hoarding was ~1000% by the Blocked Wheel hamsters compared with their *ad libitum*-fed counterparts at 0–1 h, with significant increases at 1–2 h, 2–4 and 4–24 h (range: ~750–1500; Ps < 0.05; Fig. 4). Food deprivation-induced increased hoarding occurred at all times through 24–48 h by the Free Wheel hamsters (range: ~750–2000%; P < 0.05; Fig. 4). The food deprivation-induced increased hoarding was most pronounced in the foraging hamsters (10 Revolutions group), with at least ~1500–3200% increases occurring with refeeding up to 4–24 h with a maximal increase (~3200%) at 2–4 h (Ps < 0.05; Fig. 4).

MTII inhibited food deprivation-induced increased hoarding during at least one time interval for all groups, but its effectiveness in doing so differed across the foraging groups and across time (Fig. 4). Specifically, this MC3/4-R agonist inhibited food deprivation-induced increased hoarding by the Free Wheel group only at 24–48 h (P < 0.05; Fig. 4), the Blocked Wheel group at 1–2 and 2–4 h (Ps < 0.05; Fig. 4) and the 10 Revolutions/pellet group only at 0–1 and 1–2 h (Ps < 0.05; Fig. 4). Surprisingly, MTII increased food hoarding in the food deprived 10 Revolutions/pellet hamsters at 48–72 h (P < 0.05; Fig. 4), the same time that it increased food hoarding in Experiment 1 (Fig. 2).

Discussion

This study was predicated on the notion that the food deprivation-induced increases in appetitive (food hoarding) and consummatory (food intake) ingestive behaviors were at least partially due to food deprivation-induced increases in circulating ghrelin concentrations that, in turn, stimulated AgRP release and antagonized MC3/4 receptors. This idea is somewhat, but not wholly, supported by the results of the present study using

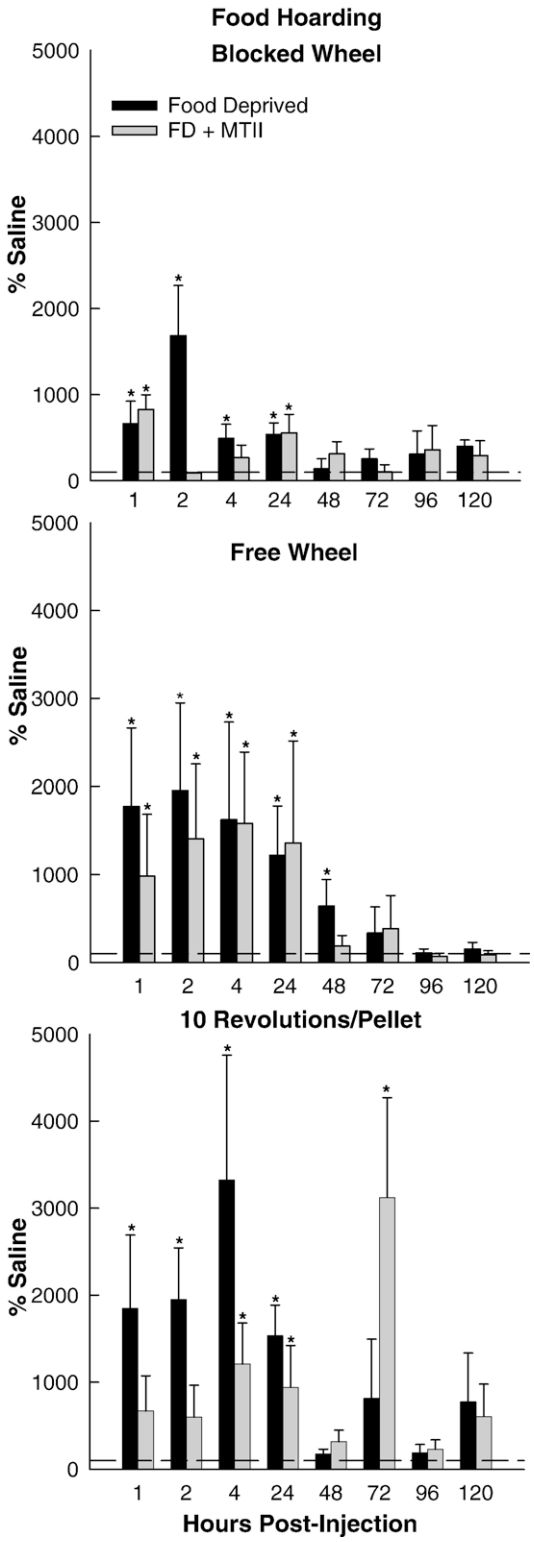

Fig. 4. Mean ± S.E.M. food hoarding as a percentage of the saline-injected *ad libitum* fed control condition for the effects of food deprivation with intracerebroventricular (icv) saline injection (black bars) and food deprivation with icv melanotan II (MTII; white bars) on hamsters without a foraging requirement and a stationary wheel (Blocked Wheel), hamsters with no foraging requirement and a freely moving wheel (Free Wheel) and hamsters with a foraging requirement (10 Revolutions/pellet). * = P < 0.05 compared to saline injected *ad libitum* fed control condition.

peripheral ghrelin injections and food deprivation to stimulate the ingestive behaviors, and the MC3/4-R agonist, MTII, to block these responses. Ghrelin-induced increases in food intake appear to act through the MC3/4-Rs in laboratory rats (Kamegai et al., 2000, 2001; Nakazato et al., 2001), and in the present study ghrelin-induced increased food intake was blocked 50% of the time across the various foraging conditions by MTII. The ghrelin-induced increased food hoarding, however, was more consistently blocked by MTII than the ghrelin-induced increased food intake. Food deprivation-induced increases in food intake, as with ghrelin, appear to act at least partially through MC3/4-R in laboratory rats (*e.g.*, Kas et al., 2003; de Rijke et al., 2005) and, in the present study, the few times food intake was elevated post fast, MTII consistently blocked this increase. Unlike the ability of MTII to block the effects of ghrelin on food hoarding, MTII was only able to attenuate fasting-induced increases in food hoarding. These data support the idea that food deprivation-induced increases in circulating ghrelin concentrations increase AgRP inverse agonism of the MC3/4-Rs.

We previously demonstrated that inverse agonism of MC3/4-R by intracerebroventricularly injected AgRP stimulates foraging and especially food hoarding, with a significantly smaller stimulation of food intake (Day and Bartness, 2004). Consistent with the notion of involvement of MC3/4-Rs in these appetitive and consummatory ingestive behaviors is the inhibition of the AgRP-triggered increase in food hoarding by prior injection of MTII (Day and Bartness, unpublished observations). Therefore, it might be expected that MC3/4-R agonism would block or at least partially inhibit, food deprivation- and ghrelin-induced increases in these ingestive behaviors in the present experiments; indeed, this was found lending further credence to the involvement of the melanocortin system in the control of food hoarding and food intake.

The effects of ghrelin on food intake were relatively short-lived in the present experiment being only significantly elevated 0–1 h post-injection, except for the hamsters foraging for their food (10 Rev Group), where food intake also was significantly increased 1–2, 2–4 and 4–24 h post injection. These data are consistent with our initial study of ghrelin effects on ingestive behaviors in this species (Keen-Rhinehart and Bartness, 2005) and the relatively early post-injection stimulation of food intake by ghrelin in laboratory rats (Wren et al., 2000; Kamegai et al., 2000; Tschop et al., 2000) and mice (Tschop et al., 2000; Asakawa et al., 2001).

MTII was effective in blocking the sporadic food deprivation-induced increases in food intake for all groups when they occurred. These data support the findings in laboratory rats and mice where this MC3/4-R agonist blocks food deprivation- (Murphy et al., 1998; Vergoni and Bertolini, 2000; Raposinho et al., 2003) and ghrelin- (Shrestha et al., 2004) induced increases in food intake. MTII was partially effective in blocking food deprivation- or ghrelin-induced increased food hoarding and this ability was highly dependent on the foraging effort. Specifically, food deprivation-induced increased food hoarding was abolished for the Blocked Wheel group at two of four intervals where it was increased (2–4 and 4–24 h, but not at 0–1 or 4–24 h), at only one of the five intervals where it was increased by Free Wheel hamsters (24–48 h), and at two out of four intervals of increased hoarding by the 10 Revolutions/pellet hamsters (0–1 and 1–2 h, but not at 2–4 or 4–24 h). By contrast, MTII was relatively effective in blocking ghrelin-induced increased food hoarding, regardless of the foraging effort. Specifically, MTII failed to block ghrelin-induced increased hoarding at only one of two intervals or increased hoarding for the Blocked Wheel group (1–2 h) and one of four intervals for the 10 Revolution/pellet group (48–72 h). The protracted elevation of food hoarding by the 10 Revolutions group (48–72 h) suggests a post-receptor mechanism, given that we found an absence of increased circulating active ghrelin 4–24 h post injection after administering the same dose of ghrelin in this species previously (Keen-Rhinehart and Bartness, 2005). Because ghrelin activates arcuate AgRP neurons (Lawrence et al., 2002) and increases its gene and protein expression (Kamegai et al., 2000, 2001), and because we previously have found that third ventricularly administered AgRP stimulates food hoarding 2–5 days post injection in Siberian hamsters (Day and Bartness, 2004), as it does for food intake in laboratory rats (Hagan et al., 2000; Lu et al., 2001), AgRP may be the mediator of the persisting effects of ghrelin on food hoarding (Keen-Rhinehart and Bartness, 2005).

These interesting, albeit sometimes complicated findings, suggest several points. The foraging effort-dependent effects of peptidergic control of food hoarding is a frequent finding in our studies of food hoarding stimulation by third ventricular injection of NPY or a Y1 or Y5 agonist (Day et al., 2005), or AgRP (Day and Bartness, 2004) or systemic injection of ghrelin (Keen-Rhinehart and Bartness, 2005). The existence of peptide effects on ingestive behavior that are dependent on the presence of an appetitive behavioral component has been reported previously. For example, icv NPY stimulates consumption of sucrose when an animal has to move to a sipper tube to drink (eat), but does not do so when the sucrose is delivered via an intraoral catheter (no appetitive response; Seeley et al., 1995). Food intake after food deprivation or ghrelin injections in the present study, and after icv NPY (Day et al., 2005) or AgRP (Day and Bartness, 2004) in our previous studies using this model, are maximal when animals are foraging for their food (10 revolutions/pellet). In laboratory rats, if the appetitive effort (bar pressing requirement) is too energetically demanding, the ability of icv NPY to stimulate food intake is less than when food is freely available (Jewett et al., 1992). These types of effects add a cautionary note to the interpretation of the roles of peptides, and likely other neurochemicals, in ingestive behaviors suggesting their effects are highly modifiable by the testing environment in the laboratory and therefore also likely highly modifiable by the environment in nature. The neural sites where MC3/4-R was acting in the present experiment are unknown and only grossly suggested by the third ventricular injections as being in periventricular hypothalamic, midbrain and/or brainstem structures.

These studies indicate that both food deprivation- and ghrelin-induced increases in food hoarding can be attenuated by MTII treatment. In addition, when MTII blocked food deprivation- and ghrelin-induced increases in food hoarding in hamsters foraging for their food (10 Revolutions/pellet group), they significantly increased their food hoarding later 48–72 h after refeeding perhaps suggesting a rebound in the behavior after its inhibition.

Collectively, the results of this study combined with known relations among food deprivation, ghrelin, NPY/AgRP arcuate nucleus neurons and food hoarding reviewed above suggest the following scenario. First, food deprivation induces increases in plasma active ghrelin (Toshinai et al., 2001; Ariyasu et al., 2001; Keen-Rhinehart and Bartness, 2005) that, in turn, stimulates arcuate nucleus NPY/AgRP synthesizing neurons (Kamegai et al., 2001; Wang et al., 2002; Cowley et al., 2003). Release of AgRP and its action at MC3/4 receptor in the hypothalamus, and perhaps other areas, stimulates circuits underlying the post-food deprivation increases in food hoarding, foraging and food intake in this species (Bartness and Clein, 1994; Wood and Bartness, 1996; Bartness, 1997; Day and Bartness, 2003). The persisting stimulation of food hoarding is likely due to AgRP-activated circuits, given that NPY administration does not produce long-lasting (>24 h) increases in food hoarding (Day et al., 2005). As noted above, the triggering of these appetitive and consummatory behaviors is not this simple, but instead involves a suite of neurochemicals and a network of circuits in addition to peripheral factors, with the present data adding to this complex picture. Specifically, because MTII was only able to attenuate fasting-induced increases in food hoarding and blocked most of ghrelin-induced increases in food hoarding, these data support the notion food deprivation increases ghrelin which increases AgRP antagonism of the MC3/4-R, and that increased ghrelin cannot be considered to have equivalent behavioral/physiological effects on these appetitive ingestive behaviors as food deprivation. Therefore, other neurochemicals must participate in the control of these appetitive behaviors. Indeed, as noted above, NPY, likely through its Y1-R subtype, is involved in foraging and hoarding of food (Day et al., 2005; Keen-Rhinehart and Bartness, 2007), at least in this species.

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