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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