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$PYY_{(3-36)}$ into the arcuate nucleus inhibits food deprivation-induced increases in food hoarding and intake☆

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ABSTRACT

Central administration of neuropeptide Y (NPY) increases food intake in laboratory rats and mice, as well as food foraging and hoarding in Siberian hamsters. The NPY-Y1 and Y5 receptors (Rs) within the hypothalamus appear sufficient to account for these increases in ingestive behaviors. Stimulation of NPY-Y2Rs in the Arcuate nucleus (Arc) has an anorexigenic effect as shown by central or peripheral administration of its natural ligand peptide YY (3-36) and pharmacological NPY-Y2R antagonism by BIIE0246 increases food intake. Both effects on food intake by NPY-Y2R agonism and antagonism are relatively short-lived lasting ~4 h. The role of NPY-Y2Rs in appetitive ingestive behaviors (food foraging/hoarding) is untested, however. Therefore, Siberians hamsters, a natural food hoarder, were housed in a semi-natural burrow/foraging system that had (a) foraging requirement (10 revolutions/pellet), no free food (true foraging group), (b) no running wheel access, free food (general malaise control) or (c) running wheel access, free food (exercise control). We microinjected BIIE0246 (antagonist) and $PYY_{(3-36)}$ (agonist) into the Arc to test the role of NPY-Y2Rs there on ingestive behaviors. Food foraging, hoarding, and intake were not affected by Arc BIIE0246 microinjection in fed hamsters 1, 2, 4, and 24 h post injection. Stimulation of NPY-Y2Rs by $PYY_{(3-36)}$ inhibited food intake at 0–1 and 1–2 h and food hoarding at 1–2 h without causing general malaise or affecting foraging. Collectively, these results implicate a sufficiency, but not necessity, of the Arc NPY-Y2R in the inhibition of food intake and food hoarding by Siberian hamsters.

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1. Introduction

In modern industrialized nations, the incidence of obesity has increased markedly over the last few decades and has led to a rise in severe secondary health consequences. Given that most animals forage for food, including humans [for reviews see: [7,31]], we postulated recently that a largely ignored set of related factors leads to sizeable food hoards and has helped propel the obesity crisis: (a) size of refrigerators, freezers and pantries, (b) processes that extend the shelf lives of food well beyond that of 25–50 years ago, and (c) ample and inexpensive calorically dense food stuffs [7]. Therefore, a deepened understanding of food foraging and hoarding may lead to behavioral and/or pharmacological treatments for overweight/obese humans, as we have suggested previously [5,7,31].

Using Wallace Craig's [14] division of animal behavior into appetitive (behavior leading to the goal) and consummatory (realization of the goal) phases, ingestive behavior is dichotomized as food foraging/hoarding (appetitive phase) and food intake (consummatory phase). We know considerably more about consummatory ingestive behaviors than appetitive behaviors because the most commonly studied animals in ingestive behavior research are laboratory rats and mice. They are not natural hoarders [for review: [7]] and are typically housed in standard cages that do not permit a significant effort to obtain food. We are able to measure food foraging, hoarding, and intake using our simulated burrow system [17] and Siberian hamsters (*Phodopus sungorus*), as they hoard food in nature [49] and in the laboratory (for review see: [7,31]).

Unlike laboratory rats and mice that overeat after a fast [e.g., [27,53]], food deprived Siberian hamsters do not overeat, nor do humans, once access to food is restored but instead 'overhoard', as do humans [for review see: [7]]. Therefore, we reasoned that other stimuli that increase food intake by laboratory rats and mice may trigger increases in food hoarding by these hamsters. Indeed, we launched several studies of the peptidergic control of food hoarding guided by this premise. Some of these studies focused on the arcuate nucleus (Arc) and the neuropeptide Y (NPY) and agouti-related protein (AgRP) neurons found therein [15,16,19,20,28,29]. As in laboratory rats [41,42,44], and mice [8], NPY and AgRP are nearly exclusively co-localized in neurons within the medial portions of the Arc in Siberian hamsters and Arc NPY and AgRP synthesis is

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stimulated by food deprivation in Siberian hamsters [22,25,34] making them a possible mediator of food deprivation-induced increases in foraging/hoarding.

NPY is a powerful orexigenic peptide when applied centrally in laboratory rats [e.g., [33,43]] and other species [for review see: [6]]. Moreover, NPY is not only a powerful orexigenic peptide in Siberian hamsters [10,15], but also is a powerful short-term (1–4 h, but up to 24 h) stimulator of food hoarding [15,16,20,28,29]. NPY has several receptor (R) sub-types (NPY-Y1-5) that are broadly distributed and their stimulation results in a diverse range of functions [for review see: [48]]. The NPY Y1- and Y5-R have been implicated in the control of food intake in laboratory rats and mice [for review see: [21]]. Microinjections of a Y1-R agonist into the PVH or PFA triggers a dose-dependent increase in food intake in laboratory rats [45] and, conversely, prior or co-injection of a NPY Y1-R antagonist into the PVH blocks the ability of PVH NPY injections to increase food intake [50,51]. NPY Y1-R agonism primarily increases food hoarding, whereas NPY Y5-R agonism primarily increases food intake in our foraging/hoarding model using Siberian hamsters [20,29].

Another NPY receptor subtype that has been strongly implicated in food intake, the NPY Y2-R, is located presynaptically and found in a number of CNS sites, including the Arc and appears to function as an autoreceptor on NPY/AgRP neurons to inhibit their activity and thereby inhibit food intake [11]. A naturally-occurring ligand for the NPY Y2-R is peptide tyrosine–tyrosine (PYY), a gut-derived hormone released from L cells in the intestine after a meal primarily in the form of $PYY_{(3-36)}$ [2]. $PYY_{(3-36)}$ is a selective agonist for the NPY-Y2R resulting in inhibition of food intake, both endogenously and exogenously [1,9]. Consistent with these effects, antagonism of the NPY-Y2R using the Y2-R selective antagonist BIIE0246, increases food intake, adding further support for a role of NPY-Y2R in the cessation of food intake [1]. Therefore, the purpose of the present experiments was to test the role of the NPY Y2-R in food foraging, food hoarding, and food intake in Siberian hamsters. To do so we asked two questions: (1) Does antagonism of NPY Y2-R using BIIE0246 increase ingestive behaviors in fed animals and (2) does agonism of NPY Y2-R using the naturally-occurring $PYY_{(3-36)}$ inhibit the food deprivation-induced increases in ingestive behaviors?

2. Materials and methods

2.1. Animals and housing

Two separate cohorts of 40 male Siberian hamsters 2.5–3 months of age and weighing 35–45 g were selected from our breeding colony. After weaning animals were group housed according to sex and raised in a long day photoperiod (16L:8D, light offset: 1900) with ad libitum access to rodent chow (LabDiet® 5001, Purina, St. Louis, MO) and tap water unless otherwise indicated. Room temperature was maintained at $21 \pm 2\,^{\circ}C$. Each cohort was treated identically. 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.

2.2. Foraging and hoarding apparatus

Animals were transferred to the foraging and hoarding room where they were singly housed in shoebox cages 290 mm × 180 mm × 130 mm (length × width × height), maintained in a 16L:8D photoperiod (light offset: 1330), and with ad libitum access to the pelleted test diet (DPPs, Purified 75 mg pellets; Bio-Serve, Frenchtown, NJ) and water. After two weeks to acclimate to the new light offset, animals were placed into the foraging and hoarding apparatus modified from Perrigio and Bronson [39] and previously described [19]. Briefly, a bottom, "burrow", cage 290 mm × 180 mm × 130 mm (length × width × height) containing Alpha-Dri bedding (Specialty Papers, Kalamazoo, MI) and one cotton nestlet (Anacare, Belmore, NY). The bottom cage was opaque and covered to simulate the darkness of a burrow. The top, "foraging", cage 456 mm × 234 mm × 200 mm (length × width × height) was equipped with a pellet dispenser, running wheel (525 cm circumference), and ad libitum access to water. The two cages were connected via convoluted polyvinyl chloride tubing (38.1 mm inner diameter and ~1.52 m long). Wheel revolutions were counted using a magnetic detection system with monitoring by a hardware/software computer interface (Med Associates, Georgia, VT). Hamsters were acclimated/trained to this apparatus for one week prior to and after cannulation (see below).

We used an acclimation/training regimen that minimizes changes in body mass and food intake that can occur when initially housed in the foraging and hoarding apparatus. Specifically, hamsters were given free access to food pellets and were able to earn a food pellet for every 10 wheel revolutions. After the first two days the free access to food was removed and all food had to be earned (1 pellet/10 wheel revolutions) for 5 d, during which body mass, wheel revolutions, pellets earned (food foraging), food intake, and food hoarding were measured daily. After the 7 d acclimation/training period, animals were placed temporarily back into the shoebox cages before cannula implantation (see below).

2.3. Foraging groups and measurement of foraging, food hoarding, and food intake

Three foraging groups were used as in our first of many reports of these groups [17]. When foraging effort is required beyond traversing the tubing, then completion of a programmed number of wheel revolutions triggers food pellet delivery, usually 10, as ≥10 inhibits hoarding due to decreased payoff–this is the 10 revolution per pellet group (10REVS). Two non-foraging conditions critical to interpreting the 10REVS foraging results were included. In the Free Wheel (FW) condition, food (300 pellets) was presented in the cage non-contingently and independent of wheel running, but wheel running was allowed (controlling for non-specific locomotor stimulation/inhibition thereby providing insight into earned food by the 10REVS group). In the Blocked Wheel (BW) condition, food (300 pellets) also was presented non-contingently, but the wheel was blocked (controlling for locomotor activity-induced changes – i.e., sedentary controls).

Foraging (pellets earned) was defined as the number of pellets earned (10REV) and food hoarding was defined as the number of pellets found in the bottom cage plus those removed from the cheek pouches. For the BW and FW groups where food was given non-contingently, food intake was defined as the number of pellets supplied (300 pellets/day) minus the total pellets hoarded or left in the top cage (surplus pellets). In the 10REV group, food intake was defined as the number of pellets earned minus the total pellets hoarded or left in the top cage (surplus pellets). The 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.

2.4. Cannula implantation, injections, and verification

Cannulae were stereotaxically implanted aimed unilaterally at the ventromedial aspect of the Arc (posterior to bregma: −1.4 mm, lateral to midline: 0.3 mm, and ventral to skull: −8.0 mm), because this region shows the densest NPY-Y2R expression in rats [37] and mice [23] under isoflurane (Aerrane, Baxter Healthcare Corporation, Deerfield, IL) inhalation anesthesia as previously described [19]. In brief, each animal had hair removed from the top of their