Role of NPY and its receptor subtypes in foraging, food hoarding, and food intake by Siberian hamsters

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Day, Diane E., Erin Keen-Rhinehart, and Timothy J. Bartness. Role of NPY and its receptor subtypes in foraging, food hoarding, and food intake by Siberian hamsters. Am J Physiol Regul Integr Comp Physiol 289: R29–R36, 2005. First published February 10, 2005; doi:10.1152/ajpregu.00853.2004.—Fasting has widespread physiological and behavioral effects such as increases in arcuate nucleus neuropeptide Y (NPY) gene expression in rodents, including Siberian hamsters. Fasting also stimulates foraging and food hoarding (appetitive ingestive behaviors) by Siberian hamsters but does relatively little to change food intake (consummatory ingestive behavior). Therefore, we tested the effects of third ventricular NPY Y1 ([Pro34]NPY) or Y5 ([Δ-Trp34]NPY) receptor agonists on these ingestive behaviors using a wheel running-based food delivery system coupled with simulated burrow housing. Siberian hamsters had 1) no wheel running access and free food, 2) running wheel access and free food, or 3) foraging requirements (10 or 50 revolutions/pellet). NPY (1.76 nmol) stimulated food intake only during the first 4 h postinjection (~200–1,000%) and mostly in hamsters with a foraging requirement. The Y1 receptor agonist markedly increased food hoarding (250–1,000%), increased foraging as well as wheel running per se, and had relatively little effect on food intake (<250%). Unlike NPY, the Y5 agonist significantly increased food intake, especially in foraging animals (~225–800%), marginally increased food hoarding (250–500%), and stimulated foraging and wheel running 4–24 h postinjection, with the distribution of earned pellets favoring eating versus hoarding across time. Across treatments, food hoarding predominated early postinjection, whereas food intake tended to do so later. Collectively, NPY stimulated both appetitive and consummatory ingestive behaviors in Siberian hamsters involving Y1/Y5 receptors, with food hoarding and foraging/running (appetitive) more involved with Y1 receptors and food intake (consummatory) with Y5 receptors.

appetitive; consummatory; wheel running; neuropeptide Y; Y1 and Y5 receptors

ANIMAL BEHAVIORIST Wallace Craig in 1918 coined the terms “appetitive” and “consummatory” for the two-part sequence of eating, drinking, and sexual behaviors (13), referring to the seeking and completion of the goal object, respectively. In terms of appetitive ingestive behavior, this consists of food acquisition (foraging) and subsequent storage for future use (hoarding; for review, see Ref. 40), whereas consummatory ingestive behavior is feeding. Considerable effort has been made toward understanding the physiological mechanisms underlying consummatory ingestive behaviors, whereas much less is known about the physiological mechanisms underlying appetitive ingestive behaviors. We have been studying the appetitive ingestive behaviors of food hoarding (2, 3, 44) and, more recently, foraging (15–17) in Siberian hamsters (Phodopus sungorus). In most species, shortfalls in food availability trigger increases in foraging and increases in food intake once food is found, whereas in hamster species, including Siberian hamsters, fasting stimulates foraging and food hoarding with minimal increases in food intake (for review, see Ref. 16). To begin to discover the mechanisms underlying appetitive ingestive behaviors, we have developed a simulated burrow housing system in the laboratory (3) to study food hoarding and recently married it to the wheel running-based foraging model of Perrigo and Bronson (34), yielding two important characteristics of foraging and hoarding in natural settings: effort and distance. Use of this housing system allows the study of both appetitive and consummatory ingestive behaviors, and in combination with a species (Siberian hamsters) that typically shows changes in foraging and hoarding largely independently of changes in food intake in response to energetic challenges [e.g., fasting (2, 3, 16)], this makes for an ideal model to study both ingestive behaviors. One candidate for the control of appetitive ingestive behavior is neuropeptide Y (NPY), an orexigenic neuropeptide produced in arcuate neurons in the hypothalamus. The ability of centrally applied NPY to stimulate food intake by laboratory rats (10, 26, 37), mice (31), and Siberian hamsters (6), as well as the suggestion that it increases appetitive ingestive behavior in rats [e.g., foraging (19, 41)], makes it a potential candidate for investigation. In addition, NPY arcuate nucleus gene expression increases with food deprivation in laboratory rats (8, 42) as well as in Siberian hamsters (29) (for review, see Ref. 4). Given the ability of food deprivation to increase food hoarding and foraging by Siberian hamsters (2, 3), it may be that centrally administered NPY would mimic the fasting-induced changes in these appetitive ingestive behaviors as well. Moreover, if NPY works in the same fashion to stimulate appetitive ingestive behaviors as it does the consummatory ingestive behavior of food intake, then it may be expected to do so via stimulation of NPY Y1 and Y5 receptor subtypes. Currently, there is a large body of evidence suggesting that these are the primary, but perhaps not exclusive, receptors underlying NPY-induced increases in feeding (20, 23, 25, 39). For those reasons, we tested the effects of NPY as well as two selective NPY receptor agonists on appetitive (foraging and food hoarding) and consummatory (food intake) ingestive behaviors in Siberian hamsters.

We asked the questions “Does centrally administered NPY stimulate the appetitive ingestive behaviors of foraging and...
NPY EFFECTS ON HOARDING AND FOOD INTAKE

Methods

Animals and Housing

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 36–42 g) were obtained from our breeding colony. The lineage of this colony was described recently (7). 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 and relative humidity was 50 ± 10%.

Hamsters were acclimated for 2 wk in specially designed hoarding apparatuses as described previously (17). Briefly, two cages were connected with a convoluted poly(vinyl chloride) tubing system (38.1 mm inner diameter and ~1.52 m long) with corners and straightways for both horizontal and vertical climbs. The top or “food” cage was 456 × 234 × 200 mm (length × width × height) and was equipped with a water bottle. The bottom or “burrow” cage was 290 × 180 × 130 mm, 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 circumference) and pellet dispenser were attached to the food cage. Wheel revolutions were counted using a magnetic detection system and monitored using a computer-based hardware-software system (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 the requisite wheel revolutions. Food hoarding (pellets hoarded) was defined as the number of pellets found in the burrow cage in addition to those removed from the cheek pouches. Surplus pellets were defined as the number of pellets removed from the food cage that were earned but neither eaten nor hoarded. For the 10 and 50 revolutions/pellet groups, food intake (pellets eaten) was defined as pellets earned minus surplus pellets minus hoarded pellets. For the free and blocked wheel groups, food intake (pellets eaten) was defined as pellets given minus pellets left in the food cage minus hoarded pellets.

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 (17). 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 pellet. This condition was in effect for 5 days during which time body mass, food intake, food hoarding, wheel revolutions, and pellets earned were measured daily. On day 8, the foraging effort was increased to 25 revolutions/pellet and remained in effect for 3 days. On day 11, the foraging effort was increased to 50 revolutions/pellet and remained in effect for 5 days. At the end of this acclimation period (16 days total), all animals were removed from the foraging apparatuses and housed in shoebox cages where food was available ad libitum with no foraging requirements. Guide cannulas were then surgically implanted in these hamsters (see Cannula Implantation). After a 1-wk postsurgery recovery period, all hamsters were returned to the hoarding/foraging apparatus and retrained to the following schedule: 5 days at 10, 3 days at 25, and 6 days at 50 revolutions/pellet.

Experimental Design

At the end of this 14-day retraining period, the hamsters were separated into four groups matched for their current body mass and average hoard size across the last 6 days of training at 50 revolutions/pellet (n = 16 per group). The four groups consisted of two foraging requirements (10 or 50 revolutions/pellet) or no foraging requirement (free wheel: an active running wheel, food freely available (activity and foraging control group), or blocked wheel: an inactive running wheel, food freely available (sedentary and nonforaging control group)). These foraging efforts were selected on the basis of our previous studies in Siberian hamsters using this hoarding/foraging system to optimize the amount of foraging and hoarding (15–17).

Each group received NPY, Y1 and Y2 receptor agonist, or the saline vehicle, and they were given in a counterbalanced schedule to control for possible order effects of peptide administration (see NPY and NPY Y1 or Y5 Receptor-Selective Agonist Administration).

Cannula Implantation

Cannulas were stereotaxically implanted into the third ventricle as described previously (15). 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 the bregma and the midsagittal sinus, and the guide cannula (26-gauge stainless steel; Plastics One, Roanoke, VA) was positioned using the following stereotaxic coordinates: level skull, anterior-posterior from bregma 0, medial-lateral from midsagittal sinus 0, and dorsal-ventral –5.5 from the top of the skull, which targeted placement just above the third ventricle. The guide cannula was secured to the skull with 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.

Intracerebroventricular Injection Protocol

The inner cannula (33-gauge stainless steel; Plastics One) extended 5.5 mm below the top of the skull. The inner cannula was connected to a microsyringe via polyethylene tubing, and the injection volume was 0.4 µl. Two hours before the onset of the dark period, food was removed from the hamsters’ pouches, and the animals were placed in clean burrow cages with access to the tubes blocked before the injection. 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. Hamsters were placed into their respective cages, and access to the tubes was restored. Food hoarding, food intake, and foraging were measured 1, 2, 4, 24, and 48 h postinjection.

Cannula Verification

After the last test, an injection of 0.4 µl of India ink 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), and their brains were removed and then postfixed in 10% paraformaldehyde for a minimum of 2 days. Each brain was sliced manually for cannula site verification. Cannulas 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 cannula placements were included in the analyses.

**NPY and NPY Y1 or Y5 Receptor-Selective Agonist Administration**

Sixty-four male hamsters were trained in the hoarding/foraging apparatus as described. Preliminary dose-response studies were conducted for NPY, the Y1 receptor agonist ([Pro34]NPY; American Peptide, Sunnyvale, CA), and the Y5 receptor agonist ([D-Trp34]NPY; a generous gift from Dr. Eric Parker (Schering-Plough Research Institute, Kenilworth, NJ)) at 0.44, 0.88, 1.76, and 3.52 nmol for all (data not shown). The 1.76-nmol dose was chosen to compare the effects of NPY with those of Y1 and Y5 receptor agonists because all ingestive behaviors tended to be maximally stimulated at this dose for each compound (data not shown). The animals were separated into four groups (n = 16 each) on the basis of baseline hoarding levels: 50 revolutions/pellet, 10 revolutions/pellet, free wheel, and blocked wheel. Equivolumetric (0.4 µl) injections of sterile saline or 1.76 nmol of either NPY or the NPY Y1 or Y5 receptor agonist were given at the beginning of the dark cycle. A within-subjects design was used, and a 3- to 4-day period of no treatment occurred between each injection (washout period) with the order of the injections done in a counterbalanced fashion. The tests continued until all animals had received NPY and the two receptor agonists as well as an associated saline vehicle for each (6 injections total). Food intake, food hoarding, and foraging were measured 1, 2, 4, 24, and 48 h postinjection (all data for the latter time period not shown because they were unaffected by the treatments).

**Statistical Analyses**

Because there was no effect of drug order, food intake, food hoarding, foraging, and wheel running, data were separately analyzed at each time interval by a two-way analysis of variance for repeated measures (2 x 4; drug x foraging group) with Bonferroni post hoc tests (GraphPad Prism v. 4.01; GraphPad Software, San Diego, CA). Differences among groups were considered statistically significant if P < 0.05. Data are presented as percentages of the saline vehicle values. Exact probabilities and test values were omitted for simplicity and clarity of presentation.

**RESULTS**

*Are Foraging, Food Hoarding, and Food Intake Stimulated by NPY or NPY Y1 or Y5 receptor-selective agonists?*

**Wheel revolutions.** Wheel running by the free wheel group, a test for locomotor activity effects of each substance, was significantly stimulated by NPY only at 1–2 h postinjection, by Y5 receptor agonist only at 4–24 h postinjection, and by the Y1 receptor agonist at all but 1–2 h postinjection (P < 0.05; Fig. 1).

**Foraging (pellets earned).** NPY increased foraging exclusively 0–1 h postinjection, especially in the 10 revolutions/pellet group (~500%, P < 0.05) and to a lesser, but significant, extent for the 50 revolutions/pellet group (~75%, P < 0.05; Fig. 2A). The Y1 ([Pro34]NPY) receptor agonist significantly increased foraging by the 50 revolutions/pellet group 0–1 h postinjection and by the 10 revolutions/pellet group at 1–2 and 2–4 h postinjection (range of increases ~120–400%, P < 0.05; Fig. 2B). Unlike both NPY and the Y1 receptor agonist, the Y5 receptor agonist only stimulated foraging at 4–24 h and only for the 10 revolutions/pellet group, but very strikingly so (~2,000%, P < 0.05; Fig. 2C, note expanded y-axis).

**Food intake (pellets eaten).** NPY increased food intake most markedly 2–4 h postinjection compared with the vehicle, especially by hamsters with a foraging requirement (10 and 50 revolutions/pellet, ~800 and 1,000%, respectively, P < 0.05; Fig. 3A). The Y1 agonist did not stimulate food intake except to a modest degree compared with NPY in the 10 revolutions/pellet group at 2–4 h postinjection (~250%, P < 0.05; Fig. 3B). The Y5 agonist significantly increased food intake compared with saline for at least one of the groups at all time points, but especially for the 10 revolutions/pellet group across the observation period (P < 0.05; Fig. 3C) and most impressively at 4–24 h postinjection (~850%, P < 0.05; Fig. 3C). The Y5 receptor agonist also significantly increased food intake for the blocked wheel and free wheel groups (1–2 h postinjection, ~200% each group; 2–4 h postinjection, ~200% blocked wheel group, P < 0.05; Fig. 3C).

![Fig. 1. Percent change in wheel running activity for the free wheel group with NPY ([Pro34]NPY); American Peptide, Sunnyvale, CA), and the Y5 receptor agonist ([D-Trp34]NPY; a generous gift from Dr. Eric Parker (Schering-Plough Research Institute, Kenilworth, NJ)) at 0.44, 0.88, 1.76, and 3.52 nmol for all (data not shown). The 1.76-nmol dose was chosen to compare the effects of NPY with those of Y1 and Y5 receptor agonists because all ingestive behaviors tended to be maximally stimulated at this dose for each compound (data not shown). The animals were separated into four groups (n = 16 each) on the basis of baseline hoarding levels: 50 revolutions/pellet, 10 revolutions/pellet, free wheel, and blocked wheel. Equivolumetric (0.4 µl) injections of sterile saline or 1.76 nmol of either NPY or the YNP Y1 or Y5 receptor agonist were given at the beginning of the dark cycle. A within-subjects design was used, and a 3- to 4-day period of no treatment occurred between each injection (washout period) with the order of the injections done in a counterbalanced fashion. The tests continued until all animals had received NPY and the two receptor agonists as well as an associated saline vehicle for each (6 injections total). Food intake, food hoarding, and foraging were measured 1, 2, 4, 24, and 48 h postinjection (all data for the latter time period not shown because they were unaffected by the treatments). Differences among groups were considered statistically significant if P < 0.05. Data are presented as percentages of the saline vehicle values. Exact probabilities and test values were omitted for simplicity and clarity of presentation.

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Food hoarding. NPY markedly stimulated food hoarding compared with saline for all groups, whether or not they were foraging for their food, and did so at all time points (range ~250–1,000%, \( P < 0.05 \); Fig. 4A), a pattern of increased food hoarding similar to that for NPY itself (Fig. 4A). Relative to NPY and the Y1 receptor agonist, the Y5 receptor agonist infrequently increased food hoarding, doing so at 0–1 h postinjection for the 50 revolutions/pellet group (\(~100\%, \ P < 0.05\) ), at 1–2 h postinjection for the free wheel and 10 revolutions/pellet groups (\(~100\% \text{ and } 200\% \), respectively, \( P < 0.05 \)) , and at 4–24 h postinjection for the 10 revolutions/pellet group (\(~500\%, \ P < 0.05 \); Fig. 4C).

**Relative distribution of pellets earned.** Because foraging was not equivalent among the groups [i.e., the absolute number of pellets earned by the 10 revolutions/pellet group was significantly greater than that for the 50 revolutions/pellet group (\( P < 0.05 \); data not shown)], a relative measure of food eaten and hoarded and of food not eaten or hoarded (surplus) was calculated [i.e., (pellets eaten/total pellets earned) \times 100]. Within the Y5 agonist groups, the 10 revolutions/pellet hamsters had significantly more surplus pellets than did the 50

![Graph A](image1)

![Graph B](image2)

![Graph C](image3)
The Y5 receptor agonist showed the most consistent effect on the relative distribution of earned pellets with a significantly greater percentage of pellets eaten than hoarded at all times, especially the 4–24 h interval (~90 vs. ~5%, respectively, for the 50 revolutions/pellet group and ~44 vs. ~27%, respectively, for the 10 revolutions/pellet group, \( P < 0.05 \); Table 1).

**DISCUSSION**

The results of the present study suggest a novel function for NPY: stimulation of two appetitive ingestive behaviors, foraging and food hoarding, in addition to its well-documented stimulation of food intake, a consummatory ingestive behavior (present experiment and Refs. 10, 26, 37). NPY significantly stimulated foraging and food intake (especially in hamsters with a foraging requirement) early postinjection and markedly stimulated food hoarding across all time periods for all groups. The Y1 receptor agonist ([Pro34]NPY) had relatively little effect on food intake compared with the Y5 receptor agonist ([d-Trp34]NPY) and NPY but markedly increased foraging and food hoarding, although some of the apparent increased foraging likely was due in part to nonspecific increases in wheel running per se (see below for details). This Y5 receptor agonist-induced increase in food intake was especially apparent in animals with a foraging requirement. The Y5 agonist also significantly increased food hoarding, but to a lesser extent than NPY and the Y1 receptor agonist. Generalizing across the foraging/nonforaging groups, NPY markedly stimulated food hoarding more than food intake and stimulated foraging more than either receptor agonist. Moreover, NPY increased food hoarding to a greater degree than either of the receptor subtype agonists, suggesting contributions of both and/or other NPY receptor subtypes to this behavior.

Wheel running by the free wheel group is an indicator of possible nonspecific effects of a treatment on locomotor activity that, if not properly interpreted, could be misconstrued as effects on foraging by hamsters where pellet delivery was contingent upon wheel running (e.g., 10 and 50 revolutions/pellet). Injections of NPY (1–2 h), the Y1 receptor agonist (all but 1–2 h) and to a lesser degree the Y5 receptor agonist (only 4–24 h) significantly increased wheel running by the free wheel group, casting some doubt as to the purity of the increased foraging by the 10 and 50 revolutions/pellet groups. Because the NPY-induced increased wheel running was confined to 1–2 h postinjection, the significant NPY-induced increased foraging by the 10 and 50 revolutions/pellet groups during the preceding period (0–1 h) appears to be bona fide increased foraging, rather than nonspecific increased locomotor activity. The same cannot be stated with confidence for the increased foraging after treatment with either agonist by these two groups because of increased wheel running in the free wheel group after the injection of either agonist. Thus centrally administered NPY clearly promotes foraging, but this effect is unlikely due to a selective stimulation of foraging by either agonist, suggesting either other or multiple NPY receptor subtype involvement. An alternative interpretation of the potentially confounding situation of agonist-stimulated wheel running and foraging is that foraging arises from an initial general increase in locomotor activity that ultimately becomes focused on food seeking. Thus increased locomotor activity

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**Fig. 4.** Percent change in food hoarded for all foraging groups given NPY (A), Y1 receptor agonist (B), and Y5 receptor agonist (C) treatment all at 1.76 nmol. Values are means ± SE. *\( P < 0.05 \) compared with saline. #\( P < 0.05 \) vs. blocked wheel. !\( P < 0.05 \) vs. free wheel. 1\( P < 0.05 \) vs. 10 Rev. 2\( P < 0.05 \) vs. 50 Rev.

**Revolution/pellets hamsters at all but the 1–2 h postinjection time period (\( P < 0.05 \); Table 1).**

NPY initially increased food hoarding early postinjection (1–2 h) compared with food intake at both foraging efforts (\( P < 0.05 \); Table 1), with food intake being significantly dominant later in the test (2–4 h postinjection, 50 revolutions/pellet; 4–24 h postinjection, 10 and 50 revolutions/pellet, \( P < 0.05 \); Table 1).

The Y1 receptor agonist triggered a cyclic pattern for eating versus hoarding in that the 50 revolutions/pellet group alternated between significantly more pellets eaten than pellets hoarded across all time periods (\( P < 0.05 \); Table 1). The pattern for the 10 revolutions/pellet group was less varied with significantly more food eaten than hoarded at 2–4 and 4–24 after Y1 agonist injections (\( P < 0.05 \); Table 1). The 50 revolutions/pellet group also had an increased percentage of hoarded pellets at 0–1 and 2–4 h postinjection compared with the 10 revolutions/pellet group.
may be an initial link in the chain of behavior that in due course becomes foraging.

Additional general support for the involvement of NPY in foraging springs forth from two independent, accidental observations in cardiovascular pharmacology, where it was noted that laboratory rats behave as though they are foraging for food during tests of the effects of centrally administered NPY on cardiovascular function (19, 41). In addition, a role for NPY in appetitive ingestive behavior also is suggested, because central administration of NPY does not stimulate sucrose intake when rats are passively fed a sucrose solution through an intraoral catheter (36). In the same animals, however, sucrose solution intake increases in response to central NPY injections if they are required to approach (appetitive ingestive behavior) the sucrose sipper tube (36). Thus these latter data indicate that the approach to the food source (appetitive ingestive behavior) is necessary for the intake-promoting effects of NPY (36). Rats also increase their visits to a sucrose source, ultimately increasing sucrose consumption, after central NPY injections (1). Together, these data and those of the present study reinforce the idea that NPY may promote the appetitive ingestive behavior of foraging as well as the consummatory ingestive behavior of eating.

NPY increased food intake in the present experiments, as has been shown repeatedly in laboratory rats and mice (10, 26, 31) and earlier in Siberian hamsters (6) as well as a wide variety of animals across several taxa (for review, see Ref. 5). The latency for the feeding effect in laboratory rats is short (~15 min; see Ref. 11), and although we do not have measures of latency in the present experiment or in the NPY-induced feeding experiment previously performed in our laboratory (6), food intake is significantly increased during the first hour compared with the saline control. Similar to the finding that the addition of a foraging requirement (approach to a sipper tube) engenders a NPY-induced increase in sucrose intake, as discussed above (36), the greatest NPY-induced increased food intake in the present experiment occurred in both foraging groups (10 and 50 revolutions/pellet). This foraging-associated enhancement of food intake by NPY in the present experiment is strikingly similar to that seen after intracerebroventricular injections of agouti-related protein (AgRP), the naturally occurring melanocortin receptor antagonist (32), in an analogous experiment with Siberian hamsters (15). Together, these examples show that orexigenic peptides such as NPY or AgRP are more potent stimulators of ingestion if the antecedent behavior to eating (foraging) is explicitly required to gain access to the food.

Beyond the initial demonstrations of the ability of NPY to increase food intake, there have been many tests trying to establish which of the six suggested NPY receptor subtypes underlie this effect (for review, see Ref. 33). Stimulation of food intake by central injections of NPY Y1 and Y5 agonists (12, 38), inhibition of NPY- and fasting-induced increases in food intake by central injections of NPY Y1 and Y5 receptor antagonists (14, 43), or injections of antisense oligonucleotides directed at these receptors (27) strongly implicate their involvement in NPY-induced hyperphagia, but without some controversy (30). Nevertheless, collectively it appears that both of these receptors are involved in NPY-induced increases in food intake in laboratory rats and mice (18). In the present study, the Y5 receptor subtype seems considerably more closely linked to feeding, especially by hamsters in the 10 revolutions/pellet and blocked wheel groups, whereas the Y1 receptor agonist had virtually no effect on food intake. Finally, as for laboratory rats, the NPY-induced stimulation of food intake is greater than that of any of the receptor agonists alone (12), suggesting the involvement of multiple NPY receptor subtypes beyond only the Y5 receptor subtype.

Although the NPY-induced increases in food intake were impressive and statistically significant (~200–1,000% vs. saline), the NPY-induced increases in food hoarding were even more so (~250–1,750%), occurring for all groups across all time periods. The magnitude of this increase frequently was greatest for the nonforaging groups (blocked and free wheel groups) and decreased with foraging effort, yet it endured through the 24-h test period, but not beyond [no change 48 h
postinjection (data not shown). In a test of NPY on food hoarding by laboratory rats (9), food hoarding was not increased. These rats, however, were subjected to progressive food restrictions to test whether NPY shifted the body weight (fat level) at which food hoarding was triggered. Food restrictions such as these should increase arcuate NPY mRNA expression and protein (35), producing increased endogenous release of NPY that, in turn, might have downregulated the NPY receptors. If this scenario occurred, then the ability of exogenous NPY to increase food hoarding would have been rendered less effective than if the rats were fed ad libitum. An alternative reason for the lack of a NPY-induced increase in food hoarding by laboratory rats is that rats do not hoard food in the wild (28). Thus the inability of NPY to stimulate a nonnaturally occurring behavior in laboratory rats might not be surprising. Regardless, the results of the present study clearly show that NPY is a potent stimulator of food hoarding in Siberian hamsters.

Central injections of the Y1 receptor agonist significantly stimulated food hoarding in most groups throughout the entire experiment, whereas the Y5 receptor agonist, although not without effect (∼200–500% increases vs. saline), only did so in the foraging groups and then not at all time points. The magnitudes of Y1 receptor agonist-induced increased food hoarding, although impressive (∼250–1,000% increases), was not as great those seen after NPY itself (a near doubling of the Y1 agonist increases). This smaller increase in food hoarding after Y1 and Y5 receptor agonist injections compared with that for NPY is reminiscent of the attenuated increases in food intake by these agonists compared with NPY in laboratory mice (21) as well as for the hamsters in the present study. Nevertheless, although stimulation of the Y1 receptor did not increase food hoarding to the same extent as NPY treatment, the present data suggest its involvement in food hoarding by Siberian hamsters.

Another way to analyze these ingestive behaviors is to calculate the percentage of earned food pellets distributed into three categories: food intake, food hoarded, and the remaining food (surplus pellets). This is an especially useful analysis when the absolute number of pellets earned differs as it did in the present study, with the 10 revolution/pellet group earning significantly more pellets than their 50 revolution/pellet group counterparts (data not shown). Analyzed in this way, the early response (1–2 h) for NPY (both groups) and the Y1 agonist (50 revolutions/pellet) is to distribute their earned pellets into food hoards, whereas all three NPY receptor agonists (NPY, Y1, and Y5) promoted eating of the earned pellets later (4–24 h). These data together with similar measures from fasting (2, 3), pregnancy (2), lactation (2), or orexigenic stimuli such as AgRP (15) or ghrelin (24) show the proclivity of hamsters for food hoarding in situations that promote profound increases in food intake by laboratory rats and mice (for review, see Ref. 4).

Finally, foraging effort affected both appetitive and consummatory ingestive behaviors. Specifically, the lower foraging requirement generated higher absolute levels of foraging and hoarding than the higher foraging requirement (data not shown). The greater foraging by hamsters with the lower foraging effort seems to make sense from a simple energetic standpoint: if there is less energy required to obtain food, then this should more readily promote foraging. The effects of foraging effort on food intake were less clear: NPY stimulated approximately the same food intake for both foraging efforts, whereas the Y5 agonist stimulated significantly greater food intake for the low compared with the high foraging effort, with the Y1 agonists showing a suggestive similar effect. The explanation for these food intake effects is not known at this time. In terms of food hoarding, NPY generally triggered a significantly higher distribution of earned pellets into food hoards at the low compared with the high foraging effort, with the Y1 agonist doing similarly at some time points and the Y5 agonist generally not affecting this measure. The attenuation of NPY-stimulated ingestive behaviors by increased foraging efforts also occurs in laboratory rats receiving NPY intracerebroventriculatively when they must bar press to earn food (22).

Collectively, the results of the present experiments suggest that NPY plays a significant role in the control of appetitive ingestive behaviors such as foraging and food hoarding, in addition to its more well-studied and accepted role in the consummatory ingestive behavior of food intake. Together, these data support the suggestion of Woods et al. (45) that ingestive behavior researchers may have overestimated the contribution of numerous neuropeptides shown to stimulate food intake in home cage feeding tests, because some of their functions may be to bring animals into contact with food, and when that occurs, feeding occurs almost reflexively. In the utopian condition of most home cage feeding tests, the appetitive phase is minimized and the consummatory phase is maximized. In contrast, when the environmental conditions allow appetitive ingestive behaviors to be expressed (i.e., when the caging system in the laboratory permits it or when it occurs naturally in the field), we likely will find an expansion of the roles of a variety of factors (e.g., neuropeptides/neurotransmitters, metabolic blockers, hormones, surgical manipulations) more traditionally thought only to affect consummatory ingestive behavior.

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REFERENCES

10. Clark JT, Kalra PS, Crowley WR, and Kalra SP.

11. Day DE and Bartness TJ.

12. Clark JT, Kalra PS, and Kalra SP.

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14. Craig W.


