ICV NPY Y1 receptor agonist but not Y5 agonist induces torpor-like hypothermia in cold-acclimated Siberian hamsters

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Pelz KM, Dark J. ICV NPY Y1 receptor agonist but not Y5 agonist induces torpor-like hypothermia in cold-acclimated Siberian hamsters. Am J Physiol Regul Integr Comp Physiol 292: 2299–2311, 2007. First published March 1, 2007; doi:10.1152/ajpregu.00790.2006.—The reduced metabolism derived from daily torpor enables numerous small mammals, including Siberian hamsters, to survive periods of energetic challenge. Little is known of the neural mechanisms underlying the initiation and expression of torpor. Hypothalamic neuropeptide Y (NPY) contributes to surviving energetic challenges by both increasing food ingestion and reducing metabolic expenditure. Intracerebroventricular injections of NPY in cold-acclimated Siberian hamsters induce torpor-like hypothermia comparable to natural torpor. Multiple NPY receptor subtypes have been identified, and the Y1 receptor and Y5 receptor both contribute to the orexigenic effect of NPY. The purpose of this research was to compare and contrast the effects of Y1 receptor activation by a specific Y1 agonist ([D-Arg25]-NPY) or Y5 receptor activation by a specific Y5 agonist ([D-Trp34]-NPY) on body temperature and subsequent food intake in cold-acclimated Siberian hamsters. Intracerebroventricular injections of Y1 agonist produced torpor-like hypothermia closely resembling that induced by intracerebroventricular NPY. The intracerebroventricular Y5 agonist infrequently produced hypothermia reaching criterion for torpor and that failed to resemble either NPY-induced or natural torpor. Combined injections of Y1 and Y5 agonists resulted in hypothermia comparable to Y5 agonist treatments alone, negating the mimicry of NPY treatment seen with Y1 agonist alone. Prior treatment with Y1 agonist or Y5 agonist surprisingly had lingering effects on NPY-induced torpor expression, Y1 agonist enhanced and Y5 agonist inhibited the effect of NPY. The ability of NPY to induce torpor-like hypothermia, especially its initiation, most likely involves activation of the NPY Y1 receptor subtype.

thermoregulation; body temperature; metabolism; neuropeptides; ingestive behavior

DAILY TORPOR IS A TACTIC USED by numerous small mammals to survive periods of energetic challenge (22, 38). Daily, or shallow, torpor is a form of reversible hypothermia in which body temperature (Tb) decreases by up to ~20°C (lower limit is Tb = ~15°C). In Siberian hamsters, for example, torpor typically is initiated early during the light (or rest/sleep) phase of the daily cycle and lasts 2–8 h (35, 48). Torpor in Siberian hamsters is primarily photoperiod dependent, occurring after ~12 wk of winter-like photoperiods with a short photophase (SP) and exposure to a low ambient temperature (Ta) (6, 24). Siberian hamsters, thus, initiate torpor when body mass (8, 40) and, more importantly, body fat reserves (7) are at their annual nadir. Torpor can also be induced in hamsters in a summer-like photoperiod with a long photophase (LP) if food availability is restricted to sufficiently reduce body mass and fat reserves (47, 48). In fact, a shortage of consumable energy is the primary cue for torpor in many other small mammalian species, regardless of the time of year (e.g., Ref. 31).

The correlation between reduced energy reserves and photoperiod-dependent torpor onset suggests feedback reflecting inadequate total fat reserves may likely be necessary for torpor initiation. Chronic serum leptin concentrations produce a relatively accurate feedback to leptin receptors in the central nervous system (CNS) as to depleted total adipose tissue reserves (1, 2, 9, 16, 20, 53). Siberian hamsters in LPs had serum leptin concentrations of 9.3 ng/ml, which were reduced to 2.1 ng/ml after 16 wk of SPs (36). Increased serum leptin concentrations, indeed, inhibit torpor expression in placentals, as well as marsupial mammals (21, 23, respectively). Reduced leptin concentrations appear to be a necessary permissive factor for torpor in Siberian hamsters; no hamster entering photoperiod-dependent torpor possessed a serum leptin concentration >2.6 ng/ml (21).

The hypothalamic ARC is among several CNS sites bearing leptin receptors (26, 28). ARC neurons possessing leptin receptors also colocalize the neurotransmitter, neuropeptide Y (NPY), and reduced leptin concentrations, as during energetic challenges, disinhibit NPY-ergic ARC neurons (e.g., Refs. 1, 3). The distinctive NPY pathway originating in the ARC terminates primarily in the paraventricular nucleus of the hypothalamus (PVH), the dorsomedial nucleus, and the medial preoptic area (4, 13, 27), which all affect thermoregulation. NPY is a very potent orexigenic transmitter, but it also actively decreases energy expenditure. Intracerebroventricular injections of exogenous NPY completely suppress neural activity in the sympathetic innervation of brown adipose tissue (BAT) (18), inhibiting nonshivering thermogenesis (55) and decreasing metabolic rate (54). In homeothermic laboratory rats, this results in a consistent decrease in Tb of 1–3°C, which falls well within the species normal range of circadian changes in Tb (12, 34, 51).

It seemed plausible that intracerebroventricular injections of NPY that produce consistent, but small, decreases in Tb of homeothermic rats might trigger much larger Tb reductions in the heterothermic Siberian hamster that, when expressing torpor, regularly undergoes Tb decreases as great as 20°C during its circadian sleep phase. Indeed, intracerebroventricular NPY treatments induced torpor-like hypothermia in ~60–65% of cold-acclimated Siberian hamsters in an LP (44), the same proportion as we typically see in photoperiod-induced torpor (Glavas M, Pelz KM, Grove KL, and Dark J; Pelz KM.
agonist ([D-Trp32]-NPY) significantly reduced interscapular intake ratio by pair-fed controls and the energy expenditure to energy ([D-Trp34]-NPY (43). Natural torpor is nearly always initiated doses of each receptor agonist on short-term and long-term experiment was undertaken to evaluate the effect of various light phase. Because we were unaware of the dose effects of 35, 48); all test injections therefore were made early during the early during the light (rest/sleep) phase of the daily cycle (e.g., Refs. 5, 42, 43). Other receptor subtypes, nevertheless, also may contribute to NPY-related food intake (e.g., Ref. 49).

Both Y1 and Y5 receptor subtypes also may be able to affect Tₜₐ and/or energy expenditure directly. In Y1 receptor KO mice, metabolic rate during the light (sleep) phase of the daily cycle was unaffected, but it was reduced by ~20% during the dark (active) phase most likely a result of the 50% reduction in locomotor activity at that time (45). Acute treatment with Y1 receptor antisense, in contrast, produced brief, transient increases in Tₜₐ without alteration in the expression of circadian rhythms (37). In addition, continuous intracerebroventricular infusion of Y1 receptor agonist ([D-Arg25]-NPY) for 5 days reduced total energy expenditure by ~20% compared with pair-fed controls and the energy expenditure to energy intake ratio by ~27%, despite a reported nonsignificant overall treatment effect (control vs. NPY vs. Y1 agonist vs. Y5 agonist) (30). Intracerebroventricular injections of Y5 agonist ([D-Trp32]-NPY) significantly reduced interscapular BAT temperature (T_BAT) and oxygen consumption, whereas Y2 and Y4 receptor agonists had no effect on either measure (32).

Both Y1 and Y5 receptor agonists may thus affect Tₜₐ and/or metabolic rate. Intracerebroventricular injected NPY induces torporlike hypothermia (44); nevertheless, the relative contribution of NPY activation by the Y1 vs. the Y5 receptor subtypes to this effect remains unknown. If the hypothermia in cold-acclimated Siberian hamsters after NPY treatments is dependent upon activation of a specific receptor subtype, then injecting an agonist specific to only that receptor subtype should be sufficient to mimic NPY-induced hypothermia. If, on the other hand, the ability of exogenous NPY to induce torporlike hypothermia involves activation at multiple receptor subtypes, then neither a Y1 specific agonist nor Y5 specific agonist will successfully mimic NPY treatment. To discriminate between these possibilities, groups of cold-acclimated Siberian hamsters in an LP were injected intracerebroventricularly with various doses of the Y1 receptor specific agonist, [D-Arg25]-NPY (42), or the Y5 receptor-specific agonist, [D-Trp32]-NPY (43). Natural torpor is nearly always initiated early during the light (rest/sleep) phase of the daily cycle (e.g., 35, 48); all test injections therefore were made early during the light phase. Because we were unaware of the dose effects of these specific receptor agonists in Siberian hamsters, an initial experiment was undertaken to evaluate the effect of various doses of each receptor agonist on short-term and long-term food intake in warm-acclimated Siberian hamsters in an LP. In laboratory rats and mice, intracerebroventricular injections of both receptor agonists induce significant increases in short-term food intake as do intracerebroventricular NPY treatments (e.g., 42, 43). In the main experiment, one group of Siberian hamsters was injected with various doses of Y1 agonist and sterile saline and a second group was injected with various doses of Y5 agonist and sterile saline in a counterbalanced design comparable to that previously used with NPY (44). All hamsters were subsequently also tested with NPY, and the Y1 agonist group was also tested with a final combined injection of the Y1 and Y5 agonists. Measures of Tₜₐ and subsequent 24 h food intake were analyzed.

MATERIALS AND METHODS

All experimental procedures were approved by the University of California Animal Care and Use Committee and conform to the guidelines set forth by the National Institutes of Health, American Physiological Society, Society for Neuroscience. The experiments were performed in research facilities approved by the Association for the Assessment and Accreditation of Laboratory Animal Care.

Animals

Sixty-nine adult female Siberian hamsters reared in a summer-like photoperiod with a long photophase (LP; 14:10-h light-dark) at Tₐ = 22°C were singly housed with Care Fresh bedding (Harlan, San Diego, CA) and provided water and food (Purina Rodent Chow #5015) ad libitum, unless otherwise stated. Twenty-four hamsters were used in experiment 1 and 43 hamsters were used in experiment 2.

General Surgical Procedure

The hamsters were deeply anesthetized by an intraperitoneal injection of a ketamine-based anesthetic at a dose of 0.34 ml/100 g body mass. The anesthetic contained 21 mg ketamine + 2.4 mg xylazine + 0.3 mg acepromazine/ml of solution. To implant a 22-gauge guide cannula directed at the third ventricle, an anesthetized hamster was placed in a stereotaxic instrument, dorsal surface of the head shaved, skin incised, and the skull made level using bregma and lambda as landmarks. A single midline trephine hole was made to allow the guide cannula to be lowered to: 0.0 mm anterior to bregma, 0.0 mm lateral to the mid sagittal line, and 5.5 mm ventral to dura. The cannula was fixed in place with stainless steel screws and dental acrylic. A steel stylet remained in the guide cannula at all times, except during injections, to maintain its patenty. To provide analgesia, 0.1 ml buprenorphine (0.015 mg/ml) was injected at the end of surgery. While deeply anesthetized, some hamsters (experiment 2 only) underwent a second procedure to place a temperature-sensitive transmitter in the abdomen. The ventral surface was shaved, the peritoneal cavity was opened, transmitter was inserted, and peritoneum and skin were closed with sterile sutures.

Neuropeptides and Intracerebroventricular Injection Procedure

The NPY Y1 receptor subtype agonist, [D-Arg25]-NPY, was injected intracerebroventricularly in doses of 2.2 µg (0.5 nmol), 6.5 µg (1.5 nmol), and 13.0 µg (3.0 nmol) Arg25 all in a 1.5-µl volume of sterile saline. The NPY Y5 receptor agonist, [D-Trp34]-NPY, was injected at doses of 2.2 µg (0.5 nmol), 6.5 µg (1.5 nmol), 13.0 µg (3.0 nmol), and 18.0 µg (4.2 nmol) Trp34 in 1.5 µl sterile saline. Control injections were 1.5 µl of sterile saline vehicle only. In addition, the animals were injected with NPY at a dose of 7.5 µg NPY/1.5 µl sterile saline. All neuropeptides were obtained from AnaSpec (San Jose, CA).
For the intracerebroventricular injection, the stylet was removed and a 28-gauge injection cannula lowered until it extended 1.0 mm beyond the tip of the guide cannula. A Hamilton microsyringe was connected to the injection cannula via polyethylene tubing and the injectant slowly infused over 30 s; the injection cannula and tubing were left in place for 30 s to allow diffusion and minimize reflex. The stylet was returned immediately upon removal of the injection cannula.

Telemetric Recording of Tḥ and Criterion for Torpor (Experiment 2 Only)

Tḥ was recorded by temperature-sensitive telemetric transmitters (Model VM-FH-LT, Minimitter, Sunriver OR) surgically implanted in each animal’s abdomen (General Surgical Procedure). Separate receiver boards under the individual cages captured the signals, which were averaged every 10 min and stored by computer (Dataquest, receiver boards under the individual cages captured the signals, which were averaged every 10 min and stored by computer (Dataquest, receiver boards under the individual cages captured the signals, which were averaged every 10 min and stored by computer (Dataquest, receiver boards under the individual cages captured the signals, which were averaged every 10 min and stored by computer (Dataquest). Individual Tḥ records were analyzed and minimum Tḥ (Tḥ min) determined after treatments. Tḥ min was defined as the lowest Tḥ during the 2 h after treatment or, in the event of torpor, the absolute lowest Tḥ achieved during torpor. The criterion for torpor was a Tḥ < 32.0°C for a minimum of 30 consecutive min. Duration of torpor was calculated as the total time from the first Tḥ < 32.0°C to the next Tḥ > 32.0°C.

Histology

With the conclusion of testing, each animal was injected with anesthesia-grade pentobarbital sodium. When deeply anesthetized, the injection cannula was lowered down the guide cannula and ~1.5 μl India ink injected to aid visualization of the injection site. The hamsters were then immediately perfused transcardially with PBS followed by buffered formalin. The brain was removed and stored in buffered formalin until sectioned through the site of the cannula track, mounted on slides, and stained with cresyl violet.

Statistics

Food intake data were analyzed with one-way repeated-measures ANOVA with post hoc comparisons between individual means where appropriate. In experiment 1, food intake was analyzed separately for each interval. Proportions of hamsters entering torpor were analyzed with Chi-square or Fisher Exact Test. Minimum Tḥ was analyzed by one-way repeated-measures ANOVA or Friedman RM ANOVA on ranks. Tḥ min during torpor and torpor duration data were analyzed with one-way ANOVA or Kruskal-Wallis one-way ANOVA on ranks. Other comparisons between two groups were analyzed with the appropriate t-test or Mann-Whitney U-Test on ranks. The relation between food intake as a function of Tvh min was tested by linear regression. All statistics were performed using SigmaStat 3.0 (SPSS). In most cases, actual test values were omitted to improve clarity of data presentation. All comparisons were considered statistically significant if P < 0.05, two-tailed tests.

Procedures

Experiment 1: Short- and Long-term Food Intake in Warm-acclimated Siberian Hamsters

After recovering from the cannula implantation surgery, the 24 hamsters were returned to the same LP and Tn and allowed several days to recover. The 24 hamsters were weighed and divided into two weight-balanced groups of 12 animals each; the first group for testing NPY Y1 agonist (Arg25) and the second group for testing NPY Y5 agonist (Trp34). On the day of testing ~1 h after onset of the light (sleep/rest) phase, all food was removed from each animal’s cage and the intracerebroventricular injections were administered. The first group received saline and three doses of Y1 agonist in a counterbalanced design over 4 days; a fifth injection of 7.5 μg NPY/1.5 μl saline was also made.

Upon completion of an intracerebroventricular injection, the animal was provided with a measured quantity of food. Food intake of each hamster was measured at 1, 2, and 24 h after treatment. Cannula placements were then histologically evaluated.

Experiment 2: Tḥ effects and long-term food intake in cold-acclimated Siberian hamsters

The 45 female Siberian hamsters were moved into an environmental chamber with the same LP but at Tn = 10°C. After 3 wk acclimation, the hamsters were removed to undergo the surgical procedure for implantation of an intracranial cannula directed at the third ventricle and an intra-abdominal telemetric transmitter as described in General Surgical Procedure. After awakening from surgery, animals were returned to the cold chamber and allowed several additional days to fully recover. The 45 hamsters were then weighed and divided into two mass-balanced groups; the first group (n = 24) for testing the Y1 agonist (Arg25) and the second group (n = 21) for testing the Y5 agonist (Trp34). All food was removed from each animal’s cage ~1 h after light onset (sleep/rest phase) and intracerebroventricular injections performed. The first group received intracerebroventricular injections of saline vehicle and three doses of Arg25 (2.2 μg, 6.5 μg, and 13.0 μg Arg25 in 1.5 μl saline) in a counterbalanced design. In a fifth intracerebroventricular injection, all hamsters received 7.5 μg NPY/1.5 μl saline. The same pattern was repeated in the second group, except that the doses of Trp34 differed from those in experiment 1. The hamsters received saline vehicle and 6.5 μg, 13.0 μg, and 18.0 μg Trp34 in 1.5 μl saline. Again, 7.5 μg NPY/1.5 μl saline constituted a fifth intracerebroventricular injection. Successive injections were always separated by at least 5 days.

On the basis of the differential experimental outcome of NPY Y1 and Y5 agonist treatments, group 1 (n = 17) was administered a sixth combined injection of both 13.0 μg Arg25 and 18.0 μg Trp34. In eight hamsters, Arg25 was injected first, and in the remaining nine hamsters, Trp34 was injected first.

For all treatments in experiment 2, the animals remained without food after treatment until the onset of the dark (active) phase, ~8–9 h (see Ref. 44). At this time, a preweighed quantity of food was provided. Food intake and body mass were measured 24 h later. At the conclusion of measurements, standard histological procedures were used to verify cannula placement. Tḥ records were analyzed after each treatment.

RESULTS

Experiment 1: Short- and Long-term Food Intake in Warm-acclimated Siberian Hamsters

Histology. All 12 of the cannulas in the NPY Y1 agonist group (Arg25) successfully reached the third ventricle with evidence of India ink, and 11 of the 12 cannulas directed at the third ventricle in the NPY Y5 agonist group (Trp34) were left in place for 30 s to allow diffusion and minimize reflux. Four successive injections were each separated by at least 5 days. The animals then received a fifth injection of 7.5 μg NPY/1.5 μl saline for comparison purposes. These procedures were repeated on the second group with 1.5 μl saline, 2.2 μg Trp34, 6.5 μg Trp34, and 13.0 μg Trp34 administered in a counterbalanced design over 4 days; a fifth injection of 7.5 μg NPY/1.5 μl saline was also made.

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doses of Y1 increased 24-h intake by ~30% over that of hamsters receiving saline or the lowest Y1 agonist dose (P < 0.05; Fig. 1B).

As expected, subsequent NPY treatment produced a statistically significant increase in food intake contrasted with saline injections during both the 0–1 and 1–2-h intervals after injection (P < 0.05, for each; Fig. 1A). Food intake did not differ between treatments during the 2–24-h interval (P > 0.05; Fig. 1A); total 24-h food intake also did not differ after saline vs. NPY treatments (P > 0.05; Fig. 1B).

**Y5 RECEPTOR AGONIST.** The Y5 agonist did not affect food intake at either the 0–1 or 1–2-h interval (P > 0.05, for both; Fig. 1C). The Y5 agonist decreased food intake during the 2–24-h interval, and 24-h intake was also decreased (P < 0.05, for both; Fig. 1C and D).

In the Y5 agonist treatment group, subsequent NPY treatment increased food intake during both the 0–1 and 1–2-h intervals after treatment (P < 0.05, for both; Fig. 1C) but decreased food intake during the 2–24-h interval (P > 0.05; Fig. 1C). Twenty-four hour food intake was comparable between saline- and NPY-treated hamsters (P > 0.05; Fig. 1D).

**Experiment 2: Tb Effects and Long-Term Food Intake in Cold-ACclimated Siberian Hamsters**

**Histology.** For the Y1 receptor agonist group, 24 Siberian hamsters had intracranial guide cannulas implanted and directed at the third ventricle. Four animals either had cannulas that missed the third ventricle or were eliminated because the cannula became dislodged before adequate data could be collected; thus, 20 hamsters with successfully implanted cannulas were used in Y1 agonist testing group. Twenty-one hamsters had indwelling guide cannulas directed at the third ventricle for the Y5 agonist group. Seven of these latter hamsters had cannulas that missed the third ventricle or became dislodged, leaving 14 hamsters for Y5 agonist treatments. All analyses are based on animals with successful cannulas.

**Tb effects. Y1 RECEPTOR AGONIST.** Intracerebroventricular injection of the Y1 receptor agonist (Arg25) produced torpor in a statistically significant proportion of the animals treated ($\chi^2 = 19.96, P < 0.05$; Fig. 2A). Hamsters typically initiated torpor within 10–30 min of Y1 agonist treatment. The highest dose of Y1 agonist (13.0 μg) induced torpor in 65% of the Siberian hamsters tested. Although Y1 agonist-induced torpor was virtually comparable to NPY-induced and natural torpor in most cases (see Fig. 3), a small number of hamsters demonstrated a slower rate of rewarming characterized by ultradian spikes in Tb after treatment with the highest dose of Y1 agonist (see Fig. 3, D and 3H).

NPY Y1 receptor agonist treatment affected Tb min (P < 0.05; Table 1). Tb min after all three Y1 agonist doses was
As dose increased (P < 0.05; Fig. 2B). The highest dose of Y5 agonist produced hypothermia in only 29% of treated hamsters. Even though T<sub>b</sub> decreases were sometimes quite profound, this hypothermia did not resemble natural torpor (Fig. 4). Both the cooling and rewarming phases were longer than during natural torpor and were often characterized by ultradian spikes of increased T<sub>b</sub>, which were sometimes quite marked (see Fig. 4, G and H).

Minimum T<sub>b</sub> was affected by NPY Y5 receptor agonist treatment (P < 0.05; Table 1). T<sub>b</sub><sub>min</sub> for all Y5 agonist doses, again, differed statistically from saline injections (Table 1). Neither T<sub>b</sub><sub>min</sub> during torpor nor torpor duration (P > 0.05, for each) was affected by Y5 agonist dose (Table 1).

**NPY TREATMENT AFTER Y1 AGONIST VS. Y5 AGONIST EXPOSURE.** The proportion of hamsters entering torpor in response to NPY treatment after being previously injected with Y1 agonist was noticeably greater than the usual proportion (95% vs. ~65%). Minimum T<sub>b</sub> of NPY treatment was statistically less than after saline injections (P < 0.05; Table 2). NPY-induced torpor in the Y1 agonist group was virtually identical to that previously observed after NPY treatment (Fig. 5, A and B), with the exception that 39% of hamsters entering torpor underwent double (i.e., two consecutive) torpor bouts (see Fig. 5, C and D).

NPY treatment after prior Y5 agonist injections, on the other hand, resulted in a lower proportion of torpor than typical (46% vs. ~65%). T<sub>b</sub><sub>min</sub> after NPY injections subsequent to Y5 treatment was less than during saline treatments (P < 0.05; Table 2). In some of the hamsters undergoing NPY-induced torpor after prior Y5 agonist injections, torpor expression appeared normal (Fig. 5, E and F). In other hamsters, it was atypical. For example, some decreased T<sub>b</sub> in a normal fashion during entry into torpor, but during rewarming, some underwent exaggerated ultradian T<sub>b</sub> cycles (Fig. 5G) and another that demonstrated brief hyperthermia before initiating the cooling phase and a slower rate of rewarming but without ultradian T<sub>b</sub> cycles (Fig. 5H). Double torpor bouts were never observed.

The differences in effect of NPY on T<sub>b</sub> after prior Y1 vs. Y5 agonist treatment were statistically significant. NPY induced torpor in a greater proportion of hamsters after prior Y1 agonist treatments than after Y5 agonist treatments (χ² = 7.30, P < 0.05; Fig. 2, A and B). In addition, minimum T<sub>b</sub> in all NPY-treated hamsters after Y1 agonist was less than after prior Y5 agonist injections (P < 0.05; Table 2). T<sub>b</sub><sub>min</sub> of just the torpid NPY-treated hamsters also was less in those previously receiving Y1 agonist vs. Y5 agonist treatments (P < 0.05; Table 2).

**COMBINED Y1/Y5 TREATMENTS.** A combined intracerebroventricular injection of the NPY Y1 and Y5 receptor agonists induced hypothermia that met our criterion for torpor in a significant proportion of the hamsters tested (Fisher’s exact test, P < 0.05; Fig. 2C). The maximum dose of Y1 agonist (13.0 µg) induced torpor in 65% of the hamsters tested. When this dose of Y1 agonist was combined with the maximum dose of Y5 agonist (18.0 µg), however, hypothermia was induced in a proportion of the animals more comparable to that of Y5 agonist treatment alone (~35% vs. ~29%, respectively). The appearance of this hypothermia did not resemble NPY-induced or natural torpor (Fig. 6).
Minimum $T_b$ was significantly reduced during Y1/Y5 combined treatments vs. saline treatment ($P < 0.05$; Table 2). $T_{b_{\text{min}}}$ of all Y1/Y5-treated hamsters and only the torpid Y1/Y5-treated hamsters was also higher than that of NPY-treated hamsters after Y1 agonist treatments ($P < 0.05$; Table 2). Both values, on the other hand, were comparable to those of hamsters receiving Y5 agonist treatments before NPY injections. In no case did torpor duration differ between groups.

**Twenty-four-hour food intake.** Y1 RECEPTOR AGONIST. Twenty-four hour food intake of Siberian hamsters was unaffected by Y1 agonist treatments ($P > 0.05$; Fig. 7A). Change in body mass during the same interval was also unaffected by treatment ($P > 0.05$; not shown). Within Y1 agonist treated hamsters, 24-h food intake was not a function of $T_{b_{\text{min}}}$ in either all hamsters ($r = -0.17$, $P > 0.05$) or only those undergoing torpor ($r = 0.03$, $P > 0.05$).
Y5 RECEPTOR AGONIST. Y5 agonist treatments did statistically affect 24-h food intake (P < 0.05), with all Y5 agonist doses decreasing food intake compared with saline treatment (P < 0.05, for each; Fig. 7B). Change in body mass during this 24 h was also statistically decreased by Y5 treatment (P < 0.05; not shown) and all three Y5 agonist doses decreased body mass greater than saline treatment (P < 0.05, for each). Again, 24-h food intake did not vary significantly as a function of T\textsubscript{b min} in either all Y5 agonist-treated hamsters (r = 0.30, P > 0.05) or only those undergoing hypothermia sufficient to reach the criterion for torpor (r = 0.20, P > 0.05).

NPY TREATMENT AFTER PRIOR Y1 VS. Y5 AGONIST INJECTIONS. NPY-treated hamsters previously receiving Y1 agonist consumed statistically less food during 24-h measurements than after saline injections (P < 0.05; Fig. 7A). After prior Y1 agonist treatments, there was a significant relation between T\textsubscript{b min} and 24-h food intake in all NPY-treated hamsters (r = 0.48, P < 0.05) but not in those undergoing torpor (r = 0.37, P < 0.05). Twenty-four-hour food intake of NPY-treated hamsters previously receiving Y5 agonist was similarly less than during saline control injections (P < 0.05; Fig. 7B). Within the previously Y5 agonist-treated hamsters, there was not a significant relation between 24-h food intake and T\textsubscript{b min} when analyzing all NPY-treated hamsters (r = 0.37, P > 0.05). Twenty-four-hour food intake, however, did vary as an inverse function of T\textsubscript{b min} in hypothermic hamsters (r = -0.84, P < 0.05), i.e., the lower the T\textsubscript{b min}, the greater the food intake, even though overall food intake was reduced.

COMBINED Y1/Y5 TREATMENTS. Although Y1 agonist treatments alone did not decrease 24-h food intake, combined Y1 and Y5 agonist treatments statistically reduced food intake (saline treatment = 6.83 ± 0.25 vs. Y1/Y5 treatment = 4.93 ± 0.50; P < 0.05). Twenty-four-hour food intake was not a function of T\textsubscript{b min} in hamsters receiving the combined treatment (r = 0.04, P < 0.05).

DISCUSSION

The NPY Y1 receptor subtype agonist, [D-Arg\textsuperscript{25}]-NPY, induced torporlike hypothermia in cold-acclimated Siberian hamsters very similar to NPY-induced torporlike hypothermia previously observed (44). NPY-induced torporlike hypothermia, in turn, very much resembles natural torpor (44). The proportion of hamsters undergoing reversible hypothermia (~65%) after Y1 agonist was comparable to that observed after NPY-induced hypothermia (44 (~63%)), and natural photoperiod-dependent torpor in this species [e.g., Glavas M, Pelz KM, Grove KL, and Dark J (64%) and Pelz KM, Routman D, Kriegsfeld LJ, and Dark J (68%), both unpublished observations]. Y1 receptor activation most accurately mimicked the initiation phase of both natural and NPY-induced torpor. In a few cases, the rewarming phase was slower than during typical torpor, and ultradian T\textsubscript{b} pulses were sometimes present, but this occurred nearly exclusively after the highest Y1 agonist dose. Limited data exist suggesting that the NPY Y1 receptor subtype may play a role in controlling T\textsubscript{b} in heterothermic laboratory rats and mice (e.g., 30, 37, 45), but the present data convincingly demonstrate that intracerebroventricular injections of Y1 agonist can, indeed, produce profound and occasionally prolonged hypothermia in the heterothermic Siberian hamster in the same manner as intracerebroventricular NPY treatments.

Even though T\textsubscript{b} was not measured, a related NPY Y5 receptor agonist, [D-Trp\textsuperscript{32}]-NPY, previously decreased interscapular BAT temperature and decreased both O\textsubscript{2} consumption and energy expenditure during the first hour after treatment in homeothermic laboratory rats (32). It, therefore, seemed likely a priori that activation of the Y5 receptor subtype would be a critical factor in the effect of NPY on torporlike hypothermia in heterothermic Siberian hamsters (44). Intracerebroventricular injection of the highly specific NPY Y5 receptor agonist, [D-Trp\textsuperscript{34}]-NPY, in the present experiment did produce hypothermia in Siberian hamsters but only occasionally did it reach the criterion for torpor. The two highest doses of Y5 agonist produced hypothermia great enough to reach our criterion for torpor in only ~29% of the treated hamsters; a proportion much less than that observed after Y1 agonist treatments, NPY injections, or during natural torpor. In addition, Y5 agonist-induced hypothermia did not resemble either natural or NPY-induced torpor. The cooling and rewarming phases were much slower than normal, and rewarming also was frequently marked by the presence of pronounced ultradian spikes in T\textsubscript{b}. Although neither the Y1 nor Y5 agonist perfectly replicated the

**Table 1. Minimum T\textsubscript{b} and torpor duration in Siberian hamsters treated with NPY Y1 receptor agonist or Y5 receptor agonist**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>T\textsubscript{b min} (°C) (All Animals)</th>
<th>T\textsubscript{b min} (°C) (Torpid Only)</th>
<th>Duration of Torpor, min</th>
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</thead>
<tbody>
<tr>
<td>Saline</td>
<td>20</td>
<td>36.2±0.2*</td>
<td>28.1±0.8</td>
<td>71.8±10.7*</td>
</tr>
<tr>
<td>2.2 μg Arg25</td>
<td>20</td>
<td>30.9±0.8†</td>
<td>27.8±0.7</td>
<td>86.0±10.2</td>
</tr>
<tr>
<td>6.5 μg Arg25</td>
<td>20</td>
<td>30.7±0.8†</td>
<td>27.1±1.0</td>
<td>134.6±25.9†</td>
</tr>
<tr>
<td>13.0 μg Arg25</td>
<td>20</td>
<td>29.7±1.1†</td>
<td>27.1±1.0</td>
<td>134.6±25.9†</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>T\textsubscript{b min} (°C) (All Animals)</th>
<th>T\textsubscript{b min} (°C) (Torpid Only)</th>
<th>Duration of Torpor, min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>14</td>
<td>36.8±0.3*</td>
<td>30.6±0.5</td>
<td>55.0±15.0</td>
</tr>
<tr>
<td>6.5 μg Trp34</td>
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<td>34.2±0.6†</td>
<td>29.2±0.4</td>
<td>122.5±36.8</td>
</tr>
<tr>
<td>13.0 μg Trp34</td>
<td>14</td>
<td>33.4±0.8†</td>
<td>30.0±0.6</td>
<td>170.0±88.0</td>
</tr>
<tr>
<td>18.0 μg Trp34</td>
<td>14</td>
<td>32.8±0.7†</td>
<td>30.0±0.6</td>
<td>170.0±88.0</td>
</tr>
</tbody>
</table>

All values are means ± SE. *Statistically significant treatment effect (ANOVA); †Statistically different from saline treatment; ‡Statistically different from 2.2 μg treatment.
effect of NPY on $T_b$ and its ability to induce torporlike hypothermia, it is very clear that activation of the Y1 receptor subtype most likely underlies the primary effect of NPY on the initiation and expression of torporlike hypothermia, especially the initial phase.

Because intracerebroventricular injection of the Y1 agonist did not always perfectly replicate comparable NPY treatment and intracerebroventricular Y5 agonist injections did produce some hypothermia, cold-acclimated Siberian hamsters were also coinjected with both the Y1 agonist and Y5 agonist. It remained possible that a more adequate mimicking of NPY treatments on torporlike hypothermia requires activation of both receptor subtypes for different aspects of torpor initiation, maintenance, and rewarming. Although Y1 agonist treatment alone nearly completely mimicked the ability of NPY to induce torpor, this effect was completely negated by concomitant Y5

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**Fig. 4.** $T_b$ records of two Siberian hamsters at $T_a = 10^\circ C$ before and after ICV treatment with saline (A, E), 6.5 μg Y5 agonist (B, F), 13.0 μg Y5 agonist (C, G), and 18.0 μg Y5 agonist (D, H). Arrowheads (*) indicate times of injection (lights on at 0500 and off at 1900).
agonist treatment. The highest doses of the Y1 and Y5 agonists individually produced torpor-like hypothermia in ~65% and ~30% of treated hamsters, respectively. Coinjection of both agonists produced criterion-reaching hypothermia in only ~35% of the hamsters, and the resultant hypothermia induced did not resemble either natural or NPY-induced torpor.

After completion of testing in the counterbalanced design for Y1 agonist effects (group 1) or Y5 agonist effects (group 2) on \( T_b \) regulation, both groups were also administered an intrace- atypical, resembling those after Y5 agonist injections (see Fig. 3). NPY after Y1 treatments appeared typical, but a few bouts were usual by reducing the proportion of hamsters entering torpor from the present study).

| Table 2. Minimum \( T_b \) and torpor duration in Siberian hamsters injected with NPY after prior Y1 agonist or Y5 agonist treatments or injected with combined Y1 + Y5 agonists |
|---|---|---|---|
| Treatment | \( n \) | \( T_{b_{min}} \) (°C) (All Animals) | \( T_{b_{min}} \) (°C) (Torpid Only) | Duration of Torpor, min |
| **NPY after Y1 agonist treatments** | | | |
| Saline | 19 | 36.2±0.2* | 25.3±0.8 | 170.6±27.6 |
| 7.5 µg NPY | 19 | 25.8±0.9 | | |
| **NPY after Y5 agonist treatments** | | | |
| Saline | 13 | 36.9±0.4* | 28.1±0.5† | 131.7±33.2 |
| 7.5 µg NPY | 13 | 31.2±0.9† | | |
| **Combined Y1+Y5 agonist treatments** | | | |
| Saline | 17 | 36.1±0.2* | 28.3±0.8§ | 116.7±29.4 |
| Y1/Y5 | 17 | 33.2±1.2‡ | | |

All values are means ± SE. *Significant treatment effect; †Significantly different from NPY after Y1 agonist treatment. ‡Significantly different from NPY after Y1 agonist treatment but not after Y5 agonist treatment.

agonist agonist treatments differentially affect NPY-induced torpor-like hypothermia are not apparent at this time—repeated intracerebroventricular NPY injections have no carryover effects (44). It is clear that the two NPY receptor subtypes activate different cellular mechanisms (5, 39, 45, 56), and in the Siberian hamster, at least, these effects can be quite protracted.

NPY Y1 receptor agonist increases short-term food intake in warm-acclimated laboratory rats (42) and the same Y1 agonist (Arg25) increased short-term food intake during the 0–1-h interval in Siberian hamsters (experiment 1). NPY Y5 receptor agonists also increase short-term food intake in warm-acclimated laboratory rats (e.g., Ref. 43), but intracerebroventricular injections of Y5 agonist in warm-acclimated Siberian hamsters had no effect on food intake during either the 0–1 or 1–2-h interval (experiment 1). There were, however, clear-cut differences in long-term food intake after intracerebroventricular injection of the Y1 agonist and the Y5 agonist. The Y1 agonist (Arg25) produced a ~30% increase in food intake after the two highest doses, whereas the Y5 agonist (Trp34) produced a ~30% decrease in food intake at the two highest doses. Y1 and Y5 agonists comparably affected short-term food intake in warm-acclimated Siberian hamsters during foraging, hoarding, and food intake measurements, but neither agonist affected long-term food intake (17), unlike the present research. The differences in food intake between the studies may be a consequence of the foraging/hoarding apparatus. Comparable testing with another ARC peptide agonist in warm-acclimated Siberian hamsters reported short- and long-term anorexigenic effects of the MC3/4 receptor agonist, MTII, in both LPs and SPs (50). The MC3/4 receptor antagonist, SHU9119, conversely, produced only limited long-term increases in food intake in both photoperiods (50). Depending on conditions and ARC peptide involved, receptor agonists and antagonists may have either short-term or long-term or both effects on food intake in Siberian hamsters (11, 17, 44, 50, present study).

Although intracerebroventricular Y1 agonist injections increased long-term food intake in warm-acclimated Siberian hamsters (experiment 1), the same treatments failed to affect 24-h food intake in cold-acclimated Siberian hamsters (exper-
iment 2), and 24-h food intake was not correlated with T_b min during treatment. This could be a ceiling effect in the cold due to the limits of food assimilation, or it could be argued that activation of Y1 receptors continues to stimulate long-term food intake in cold-acclimated Siberian hamsters, but this effect is obscured by the energy savings accruing from the high proportion of animals entering torpor and/or moderately decreasing their T_b (see NPY data discussion below). Intracerebroventricular Y5 agonist injections, on the other hand, significantly decreased 24-h food intake in cold-acclimated Siberian hamsters (experiment 2) as they had in warm-acclimated hamsters (experiment 1). The significant reduction in long-term food intake did not represent an energy savings because body mass also significantly decreased relative to saline treatments, suggesting a state of negative energy balance. The reduced food intake, in other words, required compensatory expendi-

Fig. 5. T_b records of four Siberian hamsters after 7.5 μg NPY ICV injection subsequent to ICV Y1 agonist treatments (A–D) and four hamsters receiving NPY injections subsequent to Y5 agonist treatments (E–H). Arrowheads (*) indicate times of injection (lights on at 0500 and off at 1900).
ture of energy stores. The most remarkable aspect of the significant decrease in 24-h food intake after Y5 agonist injection is that it occurs after access to food has been withheld for at least 8 h. Direct injection of NPY into the PVH increased food intake after a 1-h delay, but both norepinephrine and muscimol injections were without effect on food intake after the same delay (10). Persistent increases in food intake also occurred when food was withheld for 6 h after either central or peripheral glucoprivation induced by insulin or 2-deoxyglucose (2DG) (19, 25, 46). The basis of such lingering effects is unknown at this time but likely involves physiological mechanisms independent of the immediate activation of consummatory behavior.

As in other species, NPY injected intracerebroventricularly increased short-term food intake 1 and 2 h after Y5 agonist injection is that it occurs after access to food has been withheld for at least 8 h. Direct injection of NPY into the PVH increased food intake after a 1-h delay, but both norepinephrine and muscimol injections were without effect on food intake after the same delay (10). Persistent increases in food intake also occurred when food was withheld for 6 h after either central or peripheral glucoprivation induced by insulin or 2-deoxyglucose (2DG) (19, 25, 46). The basis of such lingering effects is unknown at this time but likely involves physiological mechanisms independent of the immediate activation of consummatory behavior.

As in other species, NPY injected intracerebroventricularly increased short-term food intake 1 and 2 h after treatment in warm-acclimated Siberian hamsters (cf. Ref. 11). Subsequent food intake apparently compensated for increased short-term intake because 24-h food intake was comparable between saline and NPY treatments.

In the present research, 24-h food intake measurements in cold-acclimated Siberian hamsters were begun with the return of food at the outset of the succeeding dark phase, ~8–9 h after treatment. Food intake was previously measured as 24 h from the time of NPY injection and thus included the immediate posttreatment time (~8–9 h) when hamsters were torpid (44). Intracerebroventricular NPY injections in cold-acclimated Siberian hamsters, nevertheless, still decreased 24-h food intake in the present experiment. We previously reported a significant carryover reduction in food intake 24–48 h after 2DG-induced torporlike hypothermia (15). Arguments can be made in favor of each method for measuring 24-h food intake: 1) 24 h from the time of treatment, thus, including the period with torpor and without food (44); vs. 2) from the time food is returned and including 24 h of food availability but not the time of torpor (present experiment). The former is probably the more logical because it does include the period (light/sleep phase) when Siberian hamsters in the field would most likely remain underground in their burrows and undergo torpor, as well as the subsequent dark-active phase.

![Fig. 6](image-url) Fig. 6. Tb records of two Siberian hamsters that received combined intracerebroventricular injections of the Y1 (13.0 µg) and Y5 (18.0 µg) receptor agonists. One (A) received the Y1 agonist (Arg25) then the Y5 agonist (Trp34) and the other (B) was injected with Trp34 before Arg25. Arrowheads (*) indicate times of injection (lights on at 0500 and off at 1900).

![Fig. 7](image-url) Fig. 7. A: effect of Y1 receptor agonist ([D-Arg²⁵]-NPY) and NPY on posttreatment 24-h food intake. B: effect of Y5 receptor agonist ([D-Trp³⁴]-NPY) and NPY on posttreatment 24-h food intake. **Statistically significant effect of Y5 agonist treatment (ANOVA). *Statistically different from saline treatment.
In summary, the torpor-like hypothermia previously induced by intracerebroventricular NPY injection (44) is apparently mediated by NPY’s activation of the Y1 receptor subtype, especially the initiation phase of torpor. ICV injections of NPY Y1 agonist produced torpor-like hypothermia in the same proportion of animals as NPY treatment, and with only a few exceptions, appeared comparable to NPY-induced and natural torpor. Although comparable intracerebroventricular injections of Y5 agonist did result in hypothermia that reached the criterion for torpor, this occurred infrequently, and the resultant hypothermia did not resemble either NPY-induced or natural torpor. In a direct test that activation of both Y1 and Y5 receptor subtypes are necessary, Y1/Y5 agonist injections did not improve the nature of the torpor-like hypothermia; in fact, just the opposite occurred. After combined injections, the frequency of torpor and its appearance were degraded from that characteristic of Y1 agonist alone to the hypothermia typical of Y5 agonist injections alone. Intracerebroventricular Y1 agonist injections appear to underlie the initiation of torpor-like hypothermia after comparable intracerebroventricular NPY injections, whereas Y5 agonist treatments do not mimic NPY treatments.

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GRANTS

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