Research Report

Neuropeptide Y induces torpor-like hypothermia in Siberian hamsters

Matthew J. Paula, David A. Freeman, Jin Ho Park, John Dark

Abstract

Intracerebroventricular (ICV) injections of neuropeptide Y (NPY) are known to decrease body temperature ($T_b$) of laboratory rats by 1–3°C. Several NPY pathways in the brain terminate in hypothalamic structures involved in energy balance and thermoregulation. Laboratory rats are homeothermic, maintaining $T_b$ within a narrow range. We examined the effect of ICV injected NPY on $T_b$ in the heterothermic Siberian hamster ($Phodopus sungorus$), a species that naturally undergoes daily torpor in which $T_b$ decreases by as much as 15–20°C.

Minimum effective dose was determined in preliminary testing then various doses of NPY were tested in cold-acclimated Siberian hamsters while food was withheld. NPY markedly reduced $T_b$ in the heterothermic Siberian hamster. In addition, the reduction in $T_b$ in 63% of the observations was sufficient to reach the criterion for daily torpor ($T_b < 32$°C for at least 30 min). Neither the incidence of torpor nor its depth or duration was related to NPY dose. Both likelihood and magnitude of response varied within animals on different test days. NPY decreased 24-h food intake and this was exaggerated in the animals reaching criterion for torpor; the decrease in food intake was positively correlated with the magnitude of the decrease in $T_b$. The mild hypothermia seen in homeothermic laboratory rats after NPY injected ICV is exaggerated, often greatly, in the heterothermic Siberian hamster. NPY treatment may be activating hypothalamic systems that normally integrate endogenous torpor-producing signals and initiate torpor.

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1. Introduction

Overwinter survival in temperate and boreal or austral environments can be a difficult energetic enterprise for many small mammals, including Siberian hamsters ($Phodopus sungorus$). Reduced ambient temperature ($T_a$) generally coincides with the lowest availability and quality of food resources, thus requiring greater foraging effort when the energetic costs of foraging are highest [63]. Several small mammals decrease metabolic loss during times of energetic challenge by employing reversible hypothermia [23]. Reversible hypothermia takes two forms: (1) hibernation or deep torpor in which body temperature ($T_b$) is reduced to as low as 2–3°C for up to 2 weeks; or, (2) shallow torpor in which $T_b$ is typically reduced for up to ~8 h and never lower than ~15°C [23,30,33]. Siberian hamsters express the latter form. When kept in a photoperiod with a short photophase, they begin to decrease food intake and body mass and undergo gonadal regression within several weeks (e.g., [32]). Photophase, however, must be decreased for ~12 weeks [25] and $T_a$ reduced before hamsters display photoperiod-dependent torpor. At this point, body mass [6,42,49] and, more importantly, fat reserves [7] are at their seasonal nadir. Similarly, Siberian hamsters in a photoperiod with a long photophase and low
$T_b$ will also express daily torpor but only if their access to food is restricted and body mass is reduced [52,53]. The primary source of thermoregulatory heat production in Siberian hamsters held in low temperatures is nonshivering thermogenesis in brown adipose tissue (BAT) [26,28,29,47], and the hypothermia during natural torpor represents a regulated temporary suspension of thermogenesis to achieve a new, lower $T_b$ setpoint [23,27].

The neurotransmitter, neuropeptide Y (NPY), plays an important role in energy balance. Of particular note is the NPY pathway originating in the arcuate nucleus of the hypothalamus and primarily terminating in the paraventricular nucleus of the hypothalamus (PVH), the dorsomedial nucleus of the hypothalamus, and the medial preoptic area (MPOA) [3,14]. NPY is a potent orexigenic agent, possibly mediating responses to marked energetic challenges. It can both acutely and chronically increase food intake (for reviews: [31,36,46,66]) and inhibit estrous behavior (e.g., [38]). It also reduces thermogenesis in the homeothermic laboratory rat. Intracerebroventricular (ICV) injection of NPY reduces metabolic rate (e.g., [62]) which is reflected in a small but reliable decrease in $T_b$ of $\sim -3 \, ^\circ C$ (e.g., [13,35,60]). The reduced metabolic rate and consequently decreased $T_b$ is most likely effected by reduced nonshivering thermogenesis; ICV injected NPY completely suppresses neural activity in sympathetic nerve fibers innervating BAT [20]. Both BAT thermogenic capacity [10,40] and thermogenic activity [5,9,62] are decreased by NPY injected ICV.

These data suggest a possible function for endogenous NPY in mediating decreases in $T_b$ within the normal range by either directly inhibiting sympathetic nervous system controlled nonshivering thermogenesis in BAT or more likely by altering the setpoint for $T_b$. This experiment tested whether ICV injection of NPY in Siberian hamsters decreases $T_b$ as it does in laboratory rats or whether NPY more dramatically produces hypothermia comparable to natural torpor. Preliminary testing was undertaken to evaluate whether several doses of NPY injected ICV that induce food intake in Siberian hamsters (1.0 $\mu$g, 2.5 $\mu$g, and 5.0 $\mu$g; Ref. [11]) would alter $T_b$. ICV injected NPY markedly increases plasma insulin concentrations when rats have access to food but not when access to food is denied [9,10]. High insulin concentrations are a correlate of increased adiposity [8] and may provide an inhibitory feedback on torpor expression in the same manner as high leptin concentrations [21]. During preliminary testing, hamsters were, therefore, tested with and without food available. In the experiment, Siberian hamsters kept in a photoperiod with a long photophase and at a low temperature (10 $^\circ C$) were injected with 5.0 $\mu$g, 7.5 $\mu$g, 10.0 $\mu$g, 15.0 $\mu$g NPY, and saline without food available, and effects on $T_b$ and subsequent food intake were monitored. We are unaware of previous studies of NPY’s effect on thermoregulation in species that undergo torpor, i.e., reversible hypothermia.

2. Materials and methods

2.1. Animals

Forty-nine adult (3–6 months old, body mass = 36.4 ± 0.8 g) female Siberian hamsters that had been raised in a photoperiod with a long photophase (14L:10D) at $T_a = 21$ $^\circ C$ were housed individually in polypropylene cages containing TekFresh (Harlan, San Diego) bedding and provided water and food (Purina Rodent Chow #5015) ad lib, unless otherwise noted.

2.2. Surgery

The hamsters underwent 2 procedures in a single surgery. To provide deep anesthesia, a ketamine-based anesthetic was injected IP at a dose of 0.34 ml/100 g body weight. The anesthetic was composed of: 21.0 mg ketamine + 2.4 mg xyazine + 0.3 mg acepromazine/ml solution. In an initial procedure to implant a single midline ICV cannula in the 3rd ventricle of the brain, each animal was placed in a stereotaxic instrument and the skin of the dorsal surface of the head was incised along the midsagittal line and retracted. With the head level, the skull was drilled and a 22-gauge cannula was lowered to the stereotaxic coordinates of 0.0 mm anterior to bregma, 0.0 mm lateral to the mid sagittal sinus, and 5.5 mm ventral to dura at a site at the top of the 3rd ventricle. Stainless steel screws and dental acrylic affixed the cannula to the skull and closed the wound; a steel stylet was inserted into the cannula to maintain patency.

In a second brief procedure while the animal remained deeply anesthetized, the midline abdomen was incised, a radiotransmitter was implanted, and the skin was closed and treated with antibiotic ointment (Pharmaderm, Manville, New York). The animals remained at $T_a = 21$ $^\circ C$ for 12–24 h to facilitate recovery from surgery. To alleviate postsurgical discomfort, 0.1 ml buprenorphine (0.015 mg/ml) was injected as an analgesic at the completion of surgery.

2.3. Telemetric recording of $T_b$ and criterion for torpor

Body temperature was recorded telemetrically via intra-abdominal transmitters (Model VM-FH or VM-FH-LT, Minimitter Co., Sunriver, OR) whose signal frequency is temperature-dependent. Receiver boards under each animal’s cage collected transmitter radio signals that were averaged over every 10-min interval and stored by computer program (Dataquest, Minneapolis, MN).

$T_b$ records were examined for the first 3 h after NPY injection for evidence of hypothermia. Minimum $T_b$ ($T_{b_{\text{min}}}$) after NPY treatment was defined as the lowest $T_b$ during this interval. If a torpor bout was initiated during this time, however, $T_{b_{\text{min}}}$ was then defined as the minimum $T_b$ achieved during torpor. Circadian variation in $T_b$ of this species ranges from 35 to 38 $^\circ C$, never decreasing below 34 $^\circ C$. Torpor was defined as a $T_b < 32$ $^\circ C$ maintained for a minimum of 30 min.
before returning to euthermia. Torpor duration was calculated as the total time that \( T_b \) remained <32 °C.

2.4. ICV injection procedure

For the injection procedure, the stylet was removed from the indwelling cannula and a 28-gauge injection cannula was slowly lowered until it projected 0.5 mm beyond the tip of the outer cannula. A Hamilton microsyringe was attached to the injection cannula via polyethylene tubing (PE 20). The injectate was slowly injected over a 30-s interval; the microsyringe was left in place for another 30 s to allow injectate diffusion before the injection probe was removed and the stylet replaced. In preliminary testing, human recombinant NPY (Sigma) was dissolved in sterile saline, and hamsters were injected with 1.0 \( \mu \)g, 2.5 \( \mu \)g, 5.0 \( \mu \)g NPY in 1.0 \( \mu \)l or 2.5 \( \mu \)l saline and 1.0 \( \mu \)l or 2.5 \( \mu \)l saline-vehicle as a control. In the experiment, hamsters were injected with 5.0 \( \mu \)g NPY/1.0 \( \mu \)l saline, 7.5 \( \mu \)g NPY/1.0 \( \mu \)l saline, 10.0 \( \mu \)g NPY/1.0 \( \mu \)l saline, and 1.0 \( \mu \)l saline. In a final test, hamsters remaining in the experiment were injected with 5.0 \( \mu \)g NPY/2.0 \( \mu \)l saline, 15.0 \( \mu \)g NPY/2.0 \( \mu \)l saline, or 2.0 \( \mu \)l saline.

2.5. Procedure

2.5.1. Preliminary testing

Twenty-six hamsters were moved into an environmental chamber with an identical photoperiod but \( T_a = 10 \) °C. After 2 weeks in the cold, the animals were removed to undergo a surgical procedure to implant an ICV cannula and a transmitter for recording \( T_b \) (see Sections 2.2 and 2.3, respectively). On the day of testing (7 days after surgery), food was removed at light onset (0530 h). Approximately 2 h after light onset (approximate time of naturally occurring torpor), 10 animals were injected with the 1.0 \( \mu \)g NPY dose. The other 10 animals were treated with 1.0 \( \mu \)l saline. Food was returned immediately after the injection procedure to half of the hamsters in each treatment group; the remaining hamsters had food withheld during testing until the subsequent onset of darkness (12–14 h without food). Water was available ad lib throughout testing. Seven days later, animals previously treated with saline received injections of 2.5 \( \mu \)g NPY and hamsters previously injected with NPY were treated with 2.5 \( \mu \)l saline; animals that previously had food returned after injection now had food withheld after treatment, and the animals that previously had food withheld now had food available during testing. Another 6 animals were injected with 2.5 \( \mu \)g NPY/1.0 \( \mu \)l, 5.0 NPY \( \mu \)g/1.0 \( \mu \)l saline, and 1.0 \( \mu \)l saline in a counterbalanced design without food available during testing.

2.5.2. Experiment

Twenty-three adult female Siberian hamsters were individually housed and moved into an environmental chamber with an identical photoperiod but \( T_a = 10 \) °C. After a minimum of 2 weeks of acclimation, they were removed from the cold to undergo a single surgery in which an indwelling cannula directed at the 3rd ventricle was implanted and a radiotransmitter for telemetrically recording \( T_b \) was placed in the abdomen.

Hamsters were weighed and divided into 4 comparable groups for testing. The animals received the 4 different treatments over 4 days of testing: 5.0 \( \mu \)g NPY, 7.5 \( \mu \)g NPY, and 10.0 \( \mu \)g NPY doses, and 1.0 \( \mu \)l saline-vehicle control treatment. Each animal received each treatment in a randomized design; for example, on the first day of testing, 1/4 received 5.0 NPY, 1/4 received 7.5 NPY, 1/4 received in 10.0 NPY, and 1/4 received saline. At least 6 but no more than 11 days elapsed between successive treatments. Because response to 5.0, 7.5, and 10.0 doses of NPY was comparable, 17 of the hamsters were administered a 5th treatment that included an additional higher dose. At least 5 days after completion of primary testing, 6 hamsters were injected with 15.0 \( \mu \)g NPY/2.0 \( \mu \)l saline, 6 were injected with 5.0 \( \mu \)g NPY/2.0 \( \mu \)l saline (2nd 5.0 \( \mu \)g NPY injection), and 5 were injected with 2.0 \( \mu \)l saline (2nd saline injection).

Shortly after the onset of the light phase (1130 h) and \(~2\) h before testing (see Preliminary testing), animals were provided clean cages and food was withheld. Water was available ad lib throughout testing. Food was returned \(~12\) h after the procedure was initiated and food intake was determined for the subsequent 12 h (24 h after treatment). The food supply was preadapted to the cold for 7 days to correct for moisture absorption.

2.6. Histology

At the end of testing, hamsters were given a lethal dose of pentobarbital sodium. When the animals were deeply anesthetized, 1 \( \mu \)l China ink was injected via the cannula to help assess cannula placement in the 3rd ventricle. The thorax was immediately opened and the animals were perfused transcardially with saline followed by 10% formalin. The brain was removed from the skull, the cannula was carefully removed, and the brain was stored in 10% formalin and 15% sucrose solution. Brains were coronally sectioned (50 \( \mu \)m) on a freezing microtome through the anterior–posterior extent of the hypothalamus and sections mounted on slides, stained with cresyl violet, and cover-slipped. Two observers without knowledge of the results determined cannula placement and presence or absence of ink in the 3rd ventricle.

2.7. Statistics

Data were analyzed with Chi-square (proportion of hamsters entering torpor), Pearson product moment correlation (correlation between body mass and \( T_b \) min), one-way ANOVA (food intake including extra observations), one-way ANOVA with repeated measures (food intake of non-
torpid + torpid hamsters and torpid hamsters only), or Kruskal–Wallis one-way ANOVA on ranks (treatment effects on $T_b$) using SigmaStat 3.0 (SPSS). Post hoc comparison of means were undertaken, where appropriate. Statistical significance was assumed with $P < 0.05$, two-tailed tests.

3. Results

3.1. Histology

Nineteen of 26 Siberian hamsters implanted with indwelling cannulae for the preliminary testing and 19 of 23 hamsters with cannulae in the experiment had cannula tracks terminating in the 3rd ventricle and evidence of ink within the 3rd ventricle (Fig. 1). Only hamsters with successful cannula placements were used in data comparisons. Effective cannula placements ranged anteriorly-posteriorly from the caudal aspect of the anterior commissure to the caudal paraventricular nucleus of the hypothalamus.

3.2. Preliminary testing

None of the 16 hamsters treated with saline or either 1.0 $\mu$g NPY (0 of 5) or 2.5 $\mu$g NPY (0 of 3) with food available exhibited $T_b < 32 ^\circ C$ (Fig. 2A). In those animals whose food was withheld during testing, no hamster treated with 1.0 $\mu$g NPY reached criterion for torpor [0 of 5 (0%); Fig. 2B], whereas several hamsters injected with 2.5 $\mu$g NPY [3 of 6 (50%); Fig. 2C] or 5.0 $\mu$g NPY [2 of 5 (40%); Fig. 2D] did reach criterion for torpor.

3.3. Experiment

NPY treatment decreased $T_b$ within 50–60 min of injection ($H = 34.03, P < 0.05$); $T_{b \min}$ of saline-treated hamsters after injection was higher than for any of the NPY treatment groups (Dunn’s Method, $P < 0.05$, for each; Table 1). The effects of NPY on $T_b$ ranged from small decreases of 0–4 $^\circ C$ to a degree of hypothermia that reached criterion for torpor (e.g., Figs. 3B–D and 4A–D), including several cases of profound torpor (Figs. 4A and D) with durations up to 19 h long. There was a statistically significant effect of NPY on $T_b$, even when the data from the hamsters reaching the criterion for torpor were excluded from calculations ($F = 8.03, P < 0.05$; not shown). Variability in magnitude of response was observed between (contrast Fig. 3 and Fig. 4) and within animals (e.g., Fig. 4).

The proportion of animals that entered torpor after treatment differed ($\chi^2 = 21.90, P < 0.05$; Table 1). Overall, 63% and 0% of animals treated with NPY and saline, respectively, reached the criterion for torpor. There was little variation between NPY doses in the proportion torpid (range: 59–67%). Similarly, neither depth of torpor ($H = 0.19, P > 0.05$; Table 1) nor torpor duration ($H = 1.38, P > 0.05$; Table 1) differed between NPY treatments.

There was no correlation between body mass and $T_{b \min}$ during NPY treatment either at the 5.0-$\mu$g ($r = 0.19, P > 0.05$), 7.5-$\mu$g ($r = 0.10, P > 0.05$), or 10.0-$\mu$g ($r = 0.02, P > 0.05$) NPY doses.

NPY treatment affected food intake when data from the original 4 groups were analyzed by repeated measures ANOVA ($F = 2.95, P < 0.05$; Table 2). NPY-treated groups consistently consumed less food than the saline-treated group, even though none of the pairwise comparisons (Holm–Sidak) reached statistical significance ($P > 0.05$ for each). The effect is even more pronounced when food intake analysis is confined to observations from the repeated measures animals that became torpid ($F = 3.24, P < 0.05$; Table 2). An analysis of the 24-h food intake including the fifth and final test injections (i.e., the 15.0-$\mu$g NPY and 0.2-$\mu$l injections of saline and 5.0-$\mu$g NPY), however, failed to reach statistical significance ($F = 1.20, P > 0.05$; Table 2).

![Fig. 1. Representative photomicrographs of a cannula implant that missed the third ventricle (top) and one in which contact was made with the third ventricle and containing evidence of ink (bottom).](image-url)
There was a positive correlation between $T_b$ min and 24-h food intake in all observations ($r = 0.45$, $P < 0.05$; not shown) as well as just those with repeated measures ($r = 0.48$, $P < 0.05$; not shown).

4. Discussion

NPY produced marked reversible hypothermia in cold-acclimated Siberian hamsters. Although a few tests produced a small effect (a decline in $T_b$ of 1 to 4°C) comparable to that observed previously in laboratory rats (e.g., [13,35,60]), in the majority of hamsters, treatment produced marked reversible hypothermia comparable to naturally occurring torpor (~10–15°C decrease in $T_b$). In addition, a few animals underwent very pronounced $T_b$ reductions of unusually prolonged duration (e.g., >18 h) outside the range observed during natural torpor (5–9 h) [30]. ICV injected NPY clearly had a much greater effect on $T_b$ in this heterothermic species than previously observed in the homeothermic rat (e.g., [13,35]). The magnitude of the decrease in $T_b$ after NPY injected ICV in mammals may be dependent on the natural range of $T_b$s observed in the species. Magnitude of circadian changes in $T_b$ in laboratory rats is 1–3°C. Circadian $T_b$ changes are normally within the same range in Siberian hamsters, except when their rest

Table 1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>$n$</th>
<th>$T_b$ min (°C), all tests</th>
<th>% Torpid</th>
<th>$T_b$ min (°C), torpid only</th>
<th>Torpor duration (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>18</td>
<td>35.8 ± 0.2**</td>
<td>0.0**</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>5.0 NPY</td>
<td>19</td>
<td>29.5 ± 1.1***</td>
<td>63.2</td>
<td>27.5 ± 1.4</td>
<td>230 ± 97</td>
</tr>
<tr>
<td>7.5 NPY</td>
<td>17</td>
<td>30.3 ± 0.9***</td>
<td>64.7</td>
<td>28.5 ± 0.9</td>
<td>107 ± 28</td>
</tr>
<tr>
<td>10.0 NPY</td>
<td>17</td>
<td>31.1 ± 0.8***</td>
<td>58.8</td>
<td>28.8 ± 2.9</td>
<td>109 ± 13</td>
</tr>
<tr>
<td>15.0 NPY</td>
<td>6</td>
<td>29.1 ± 2.5***</td>
<td>66.7</td>
<td>26.3 ± 2.9*</td>
<td>230 ± 148*</td>
</tr>
</tbody>
</table>

* Underestimates, because one value in each cell is based on last recorded $T_b$ when transmitter ceased functioning during torpor entrance.
* Mean ± SE.
** Statistically significant treatment effect ($P < 0.05$).
*** Statistically different from saline ($P < 0.05$).
Fig. 3. $T_b$ records of an individual Siberian hamster at $T_a = 10\, ^\circ\text{C}$ before and after treatment with saline (A), 5.0 $\mu$g NPY (B), 7.5 $\mu$g NPY (C), and 10.0 $\mu$g NPY (D). Asterisks (*) indicate time of injection (lights on at 1130 h).

Fig. 4. $T_b$ records of an individual Siberian hamster at $T_a = 10\, ^\circ\text{C}$ before and after treatment with 5.0 $\mu$g NPY (A), 7.5 $\mu$g NPY (B), 10.0 $\mu$g NPY (C), and 15.0 $\mu$g NPY (D). Asterisks (*) indicate time of injection (lights on at 1130 h). (Note change in scale of Time of Day values on the abscissa in panels A and D.)
phase decrease in $T_b$ becomes greatly exaggerated during natural torpor and $T_b$ may be reduced by as much as 20 °C.

All doses of NPY above the minimally effective dose produced reversible hypothermia in nearly the same proportion of hamsters tested; approximately 63% of animals evidenced torpor-like hypothermia after each of the 4 NPY doses. The 15.0-μg NPY group represented only 6 observations, making its proportion somewhat less reliable; nevertheless, it would appear that, at effective doses, NPY’s torpor-like effect in Siberian hamsters may be an all-or-none event. Some hamsters never evidenced sufficient hypothermia to reach criterion for torpor and some responded on 1 day but not on others, regardless of dose. These findings are reminiscent of natural torpor. A substantial proportion of Siberian hamsters challenged with low $T_b$s and either a short photophase or food restriction never undergo natural torpor and those undergoing torpor do not do so everyday (e.g., [21,55]). The similarities in frequency of photoperiod- and NPY-induced torpor lead one to speculate that the hamsters responding to NPY with torpor-like hypothermia are the same animals that undergo photoperiod-induced torpor and the remaining cohort of hamsters fails to exhibit torpor after either prolonged winter-like conditions or NPY treatment. The factor that keeps some Siberian hamsters from entering torpor, whether natural or induced, is unknown. The variability in response to NPY between and within animals in the present experiment may, thus, reflect inherent intraspecific differences in propensity to display torpor.

Cellular glucoprivation after systemic injection of the non-metabolizable glucose analogue, 2-deoxy-D-glucose (2DG [65]) produces a mild hypothermia (~1–2 °C) in mice, rats, and humans comparable to that seen after NPY injected ICV in laboratory rats [22,58,59]. Systemic 2DG treatment of Siberian hamsters, on the other hand, produces a pronounced reversible hypothermia ($T_b$ ~20 °C) that resembles both natural torpor [17,18] and the NPY-induced hypothermia in the present experiment. Although 2DG did not induce hibernation or even mild hypothermia in golden-mantled ground squirrels, a placental hibernator [16], 2DG did produce marked hypothermia in a marsupial hibernator, Cercartetus nanus, that represented a change in $T_b$ setpoint [64]. Systemic 2DG in Siberian hamsters produces torpor-like hypothermia within 60 min of treatment and in 60–70% of treated hamsters, both, as in the current research. In laboratory rats, systemic 2DG treatment blocks sympathetic nervous system activation of interscapular BAT [19] in the same manner as NPY injected ICV [20]; sympathetic innervation provides the primary neuroendocrine activation of BAT nonshivering thermogenesis (e.g., [19]). Although 2DG may be mimicking the effect of natural metabolic/endocrine signals instigating torpor and NPY may be acting more downstream in the torpor mechanism, 2DG and NPY, thus, may both eventually alter $T_b$ via a common thermoregulatory effector system.

Although there are several NPY pathways in the brain, the NPY pathway originating in the arcuate nucleus and terminating primarily in the paraventricular nucleus of the hypothalamus (PVH), the dorsomedial nucleus of the hypothalamus, and the medial preoptic area (MPOA) is most likely part of the neural circuitry that generates hypothermia during NPY-induced (and 2DG-induced) torpor in the Siberian hamster. This pathway appears critical for several reasons. Firstly, all 4 nuclei are circumventricular structures and candidate feedback sites for blood-borne metabolic factors signaling energetic challenge (e.g., glucose, leptin, insulin, etc.; e.g., [8]). Secondly, direct injection of NPY into both the MPOA and PVH in rats mimics the effect of NPY injected ICV on $T_b$ [15,34,35,51]. During preliminary testing, one hamster’s cannula terminated in the PVH rather than the 3rd ventricle. This hamster underwent torpor-like hypothermia ($T_b$ min = 25.3 °C) after a 2.5-μg NPY injection. Lastly, systemic 2DG treatment that similarly eliminates sympathetic neural activity to BAT [19] may act via this pathway because 2DG increases both arcuate nucleus NPY mRNA and NPY contents [2,57]. If arcuate NPY-ergic pathways underlie NPY’s (and 2DG’s) torpor-inducing effect, then it remains possible that this pathway may also function during initiation of naturally occurring torpor.

There are several possible ways in which NPY could be producing torpor-like hypothermia. NPY injected ICV could be simply disrupting ability to thermoregulate, such as, directly inhibiting central mechanisms controlling nonshivering thermogenesis in BAT. The PVH and MPOA are intimately involved in sympathetic outflow to BAT [4], and
intracranial NPY inhibits sympathetic neural input to BAT [20]. A simple suspension of BAT thermogenesis, however, is unlikely to explain the torpor-like hypothermia after ICV injected NPY, especially those cases of profound and protracted reversible hypothermia (see, Fig. 4). There are numerous non-BAT thermogenic mechanisms (e.g., shivering) that should be capable of compensating for an acute loss of BAT nonshivering thermogenesis and defending an euthermic $T_b$ in cold-acclimated Siberian hamsters. For example, chronically compromising BAT function either by a 40% surgical reduction in BAT mass [26] or by transgenic reduction in BAT uncoupling protein (UCP 1) concentrations [39] fails to compromise euthermia. It seems more likely, therefore, that NPY injected ICV is directly affecting $T_b$ setpoint, resetting it to a new lower value. NPY may, thus, be activating the same neural circuitry that normally mediates expression of naturally occurring torpor by initiating a comparable shift in $T_b$ setpoint. Although such a role for NPY-ergic mechanisms is only speculative at this time, the MPOA interestingly mediates the $T_b$ setpoint change during deep torpor (see, [23]) and the arcuate nucleus NPY pathway has terminations in the MPOA.

The powerful orexigenic action of NPY is well-established in a broad range of species (e.g., [12,41,50]), including Siberian hamsters [11]. ICV injected NPY in warm-acclimated Siberian hamsters markedly increases short-term food intake ([11], Pelz and Dark, unpublished observations), but has no effect on 24-h food intake (Pelz and Dark, unpublished observations). In the present experiment, food intake measured 24 h after NPY treatment was significantly reduced compared to saline-treated hamsters. Because food was withheld until ~10–12 h after treatment, food intake measurements did not overlap with torpor with the exception of 1 animal with a torpor bout >18 h. Food intake subsequent to NPY treatment was positively correlated with the decrease in $T_b$ min, and this effect was exaggerated when the comparison was limited to only those animals undergoing sufficient hypothermia to reach criterion for torpor. Food intake during the 24 h following NPY-induced torpor was reduced by ~30%. Rather than a direct action on neural eating mechanisms, the suppressive effect of NPY on food consumption is more likely an indirect effect due to reduced metabolism during torpor and the resulting reduction in 24-h energy expenditure. Torpor expression is presumably adaptive because it reduces energy requirements. Indeed, reduced metabolic activity during natural torpor provides a considerable energy savings (~25% in Siberian hamsters; Ref. [54]) and consequently a reduced need for aboveground foraging in winter. The occurrence of torpor during the sleep phase significantly decreases 24-h food intake in Siberian hamsters, whether it is naturally occurring, 2DG-induced, or NPY-induced torpor ([18,37,54], present experiment).

None of the Siberian hamsters allowed access to food after NPY treatment in the preliminary study expressed torpor. Presumably, it is not the presence of food per se that inhibits NPY-induced torpor, because Siberian hamsters in laboratory routinely undergo photoperiod-dependent torpor in the presence of food. Concomitant food availability and NPY treatment leads to pronounced increases in circulating insulin, but NPY treatment without food available does not increase insulin concentrations [9,10,45]. Timing of photoperiod-dependent torpor onset (~12–15 weeks) is correlated with minimal body and white adipose tissue (WAT) mass (e.g., [25]) as well as minimal plasma insulin concentrations [42–44]. Although only correlative, the data suggest that elevated insulin concentrations may counteract torpor expression. Insulin provides a positive feedback signal to the central nervous system reflecting WAT mass [8,56], as does leptin [1,48,61]. Elevated leptin concentrations, which inhibit NPY release, also antagonize torpor expression [21,44]; markedly reduced leptin concentrations may be necessary, but not sufficient, for torpor occurrence in Siberian hamsters (see, [21]). Feedback from depleted WAT reserves is signaled by minimal leptin, and possibly insulin, concentrations which may in turn release from chronic inhibition central NPY mechanisms that underlie torpor expression.

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