Monosodium glutamate-induced arcuate nucleus damage affects both natural torpor and 2DG-induced torpor-like hypothermia in Siberian hamsters

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Pelz KM, Routman D, Driscoll JR, Kriegsfeld LJ, Dark J. Monosodium glutamate-induced arcuate nucleus damage affects both natural torpor and 2DG-induced torpor-like hypothermia in Siberian hamsters. Am J Physiol Regul Integr Comp Physiol 294: R255–R265, 2008. First published March 1, 2007; doi:10.1152/ajpregu.00387.2007.—Siberian hamsters (Phodopus sungorus) have the ability to express daily torpor and decrease their body temperature to ~15°C, providing a significant savings in energy expenditure. Daily torpor in hamsters is cued by winterlike photoperiods and occurs coincident with the annual nadirs in body fat reserves and chronic leptin concentrations. To better understand the neural mechanisms underlying torpor, Siberian hamster pups were postnatally treated with saline or MSG to ablate arcuate nucleus neurons that likely possess leptin receptors. Body temperature was studied telemetrically in cold-acclimated (10°C) male and female hamsters moved to a winterlike photoperiod (10:14-h light-dark cycle) (experiments 1 and 2) or that remained in a summerlike photoperiod (14:10-h light-dark cycle) (experiment 3). In experiment 1, even though other photoperiodic responses persisted, MSG-induced arcuate nucleus ablations prevented the photoperiod-dependent torpor observed in saline-treated Siberian hamsters. MSG-treated hamsters tended to possess greater fat reserves. To determine whether reductions in body fat would increase frequency of photoperiod-induced torpor after MSG treatment, hamsters underwent 2 wk of food restriction (70% of ad libitum) in experiment 2. Although food restriction did increase the frequency of torpor in both MSG- and saline-treated hamsters, it failed to normalize the proportion of MSG-treated hamsters undergoing photoperiod-dependent torpor. In experiment 3, postnatal MSG treatments reduced the proportion of hamsters entering 2DG-induced torpor-like hypothermia by ~50% compared with saline-treated hamsters (38 vs. 72%). In those MSG-treated hamsters that did become hypothermic, their minimum temperature during hypothermia was significantly greater than comparable saline-treated hamsters. We conclude that 1) arcuate nucleus mechanisms mediate photoperiod-induced torpor, 2) food-restriction-induced torpor may also be reduced by MSG treatments, and 3) arcuate nucleus neurons make an important, albeit partial, contribution to 2DG-induced torpor-like hypothermia.

thermoregulation; leptin; neuropeptide Y; body mass; fat

SIBERIAN HAMSTERS UNDERGO numerous physiological and behavioral changes when experiencing a winterlike photoperiod with a short photophase (SP) and low ambient temperatures (Ta), including bouts of shallow, daily torpor (e.g., Ref. 23). Daily torpor is a form of reversible hypothermia that occurs during the rest/sleep phase of the circadian cycle, coincident with the time of the circadian minimum in body temperature (Tb). In fact, it is accepted that this represents an exaggeration of the usual 1–3°C sleep-dependent decrease in Tb (13, 52). During daily torpor, however, Tb may decrease by as much as ~20°C for 5–8 h (27, 43). Siberian hamsters expressing torpor typically do so 2–3 times a week (43). Expression of daily torpor generates a significant savings in energy expenditure, thus decreasing energy intake requirements in winter (21, 41).

Although most photoperiodic responses (e.g., reduced food intake and reproductive regression) are initiated within several weeks of the onset of a winterlike photoperiod, torpor is not initiated for ~12 wk (e.g., Ref. 23). This time coincides with the nadir in body mass (4, 6, 42) and minimal white adipose tissue reserves (5). Reduced fat reserves appear necessary for photoperiod-dependent daily torpor because high leptin concentrations suppress torpor expression (19, 20, 22). Only Siberian hamsters with markedly reduced serum leptin concentrations (<2.6 ng/ml) enter torpor, suggesting that chronically low leptin concentrations are a permissive factor for torpor onset (19).

Reduced leptin concentrations are required for the exaggerated circadian decrease in Tb during daily torpor, and ob/ob mice, which are leptin deficient, undergo a significantly greater decrease in circadian Tb min than do lean mice. Even though food restriction decreases Tb min in both lean and ob/ob mice, it is reduced to a greater degree in ob/ob mice (28). Exogenous leptin treatment, on the other hand, prevents food restriction from decreasing Tb min in lean mice (14). Suckling-age rat pups undergo similar decreases in light/rest phase Tb and metabolic rate. This decrease is due to a temporary shutdown of sympathetic mediated nonshivering thermogenesis in brown adipose tissue (BAT) (33, 38) and, as in adult animals, is blocked by leptin treatments (48). Repeated postnatal monosodium glutamate (MSG) treatments produce a specific pattern of neural degeneration primarily focused in the hypothalamic arcuate nucleus (ARC) (e.g., Refs. 7 and 26), especially targeting ARC neuropeptide Y and proopiomelanocortin neurons, which both colocalize leptin receptors but have opposing effects on metabolism (9, 26, 31). MSG ablation of the ARC eliminates “torpor-like” circadian decreases in Tb in suckling rats (45).

The purpose of the present series of studies was to determine whether MSG-induced ablation of ARC mechanisms would eliminate photoperiod-dependent torpor, whose onset appears dependent upon chronically reduced leptin feedback as a permissive factor. ARC MSG lesions have been successfully produced in Siberian hamsters, demonstrating that SP-induced changes in hamster body mass, food intake, fur color, and testis mass persist after ARC ablation (16). If MSG ablations in Siberian hamsters mimic the effect on circadian Tb min in suckling rat pups (45), then ARC MSG ablations should eliminate photoperiod-dependent torpor in hamsters. Differ-
ences in fat reserves between MSG-and saline-treated hamsters raised in SPs were previously nonsignificant (16). Nevertheless, to eliminate the possibility that any reduced frequency of torpor in MSG-treated hamsters may be due to enlarged fat reserves and, hence, greater leptin concentrations, a second experiment was undertaken. Occurrence of torpor in SP-exposed Siberian hamsters was also tested after food restriction to determine whether reducing body fat would normalize torpor frequency.

The nonmetabolizable glucose analog, 2-deoxy-D-glucose (2DG), disrupts cellular glucose oxidation, producing glucoprivation (e.g., Ref. 54). Large doses of 2DG (2,500 mg/kg body mass) induce torpor-like hypothermia in both Siberian hamsters, a placental mammal (11), and Cercartetus nanus, a hibernating marsupial mammal (53). In both species, hypothermia is a regulated decrease in $T_b$ setpoint whose depth and duration are characteristic of daily torpor (10, 53), even though the latter species is a hibernator. Because 2DG-induced hypothermia and natural torpor are both regulated changes in $T_b$ with numerous common characteristics, it is likely they share a common mechanism despite possessing different triggering mechanisms. For this reason, hypothermia was examined following systemic 2DG injections in MSG- and saline-treated Siberian hamsters in a third experiment.

MATERIALS AND METHODS

This research was performed in research facilities approved by the Association for the Assessment and Accreditation of Laboratory Animal Care. All experimental procedures were reviewed and approved by the University of California Animal Care and Use Committee and conform to guidelines established by the National Institutes of Health.

Animals

Ninety-two adult male and female Siberian hamsters reared in an LP at $T_a = 22 \pm 1 ^\circ C$ were used. All hamsters were singly housed at weaning in polypropylene tub cages with Care Fresh bedding (Harlan, San Diego, CA) and were provided food (Purina Rodent Chow #5015) and water ad libitum.

Monosodium Glutamate-Induced Arcuate Nucleus Ablation

Siberian hamster dams and their newborn litters were assigned to the study, and individual pups were randomly assigned to the MSG or saline treatment groups and differentially marked with permanent ink for identification. As litters were assigned, an attempt was made to keep the number of males and females balanced.

MSG solution (L-glutamic acid monohydrate sodium, Sigma-Aldrich, St. Louis, MO; 80 mg/ml sterile saline) was used as described by Ebling et al. (16) for Siberian hamsters. MSG-treated hamsters were injected subcutaneously with 4 mg MSG/g body mass, and saline-treated hamsters were injected with 0.05 ml sterile saline/g body mass on postnatal days 4, 5, 6, and 7. At weaning, pups were housed individually and remained in an LP at 22°C.

2DG Treatment

2DG (Sigma-Aldrich) was used at a dose of 2,500 mg/kg body mass. Adult male and female Siberian hamsters ($n = 59$) received intraperitoneal injections of 2.5 mg 2DG/g body mass as a solution of 2.5 mg 2DG/0.02 ml sterile saline. They also received control injections of 0.02 ml saline/g body mass in a counterbalanced design.

Measuring $T_b$ and Criterion for Torpor

To measure $T_b$, hamsters underwent a brief surgical procedure under a ketamine based anesthesia [0.34 ml solution/100 g body mass (21 mg ketamine + 2.4 mg xylazine + 0.3 mg acepromazine/ml)]. The midline abdomen was shaved and incised and a radiotransmitter for telemetric recording of $T_b$ (Model VM-FH-LT, Minimitter, Sunriver OR) was inserted into the abdomen. The peritoneum and skin were closed with sterile sutures. At the end of surgery, hamsters were injected with 0.1 ml buprenorphine (0.015 mg/ml) as an analgesic to alleviate postsurgical discomfort.

Each animal’s cage was placed on a separate receiver board, and $T_b$ was averaged and recorded every 10 min (Dataquest, St. Paul, MN). Individual $T_b$ records were examined after treatments and minimum $T_b$ ($T_{b\min}$) was defined as the lowest $T_b$ observed during the immediate 2 h after treatment or the absolute minimum $T_b$ observed if torporlike hypothermia occurred. Torpor was defined as a $T_b < 32.0 ^\circ C$ for 30 consecutive min (36, 42), and torpor duration was defined as total time from 1st $T_b < 32.0 ^\circ C$ to 1st $T_b > 32.0 ^\circ C$.

Fur Color Index

To provide verification of photoperiodic responding in MSG- and saline-treated Siberian hamsters, fur (pelage) was rated every 1–2 wk using a four-stage color scale developed for moultling Siberian hamsters (15).

Histological Analysis

For immunohistochemical analysis of neuropeptide Y (NPY), hamsters were given a lethal dose of pentobarbital sodium, and when deeply anesthetized, they were transcardially perfused with 0.1 M PBS followed by 4% paraformaldehyde buffered with PBS. Brains were postfixed in 4% paraformaldehyde in PBS for 2 h before being cryoprotected overnight in a 25% sucrose solution in PBS. Because of the excessive number of hamsters involved in this research (90+) and the well-established outcome of postnatal MSG treatments (e.g., Refs. 16 and 55), immunohistochemical procedures were undertaken on only a sampling of the MSG-treated ($n = 28$) and saline-treated ($n = 8$) hamsters. Brains were sectioned in the coronal plane at 40 µm using a cryostat. Alternate sections were collected into PBS (pH 7.4) to be processed for NPY. Tissue was submersed in 0.5% hydrogen peroxide to reduce endogenous peroxidase activity. Subsequently, samples were incubated in 10% normal goat serum (Vector Laboratories, Burlingame, CA) at room temperature for 1 h. Following preincubation in normal goat serum, the sections were incubated for 48 h at 4°C in rabbit anti-NPY (ImmunoStar, Hudson, WI) and diluted 1:8,000 in PBS with 0.3% Triton X-100 (Sigma). Sections were then mounted on gelatin-coated slides, dehydrated in a graded series of ethanol solutions (30, 70, 95, and 100%), and cleared in Xylenes (Fisher Scientific, Pittsburgh, PA) before the application of coverslips. Slides were examined for NPY immunoreactivity using a Zeiss Z1 microscope.

Optical Density Measurement

The optical density of NPY fiber staining in the ARC and paraventricular nucleus of the hypothalamus (PVN) was measured. Each pixel in the grayscale image capture has a measurable specific intensity, with each pixel having a value ranging from 0 (white) to 256 (black). The average value for all pixels in an outlined area is taken as the mean intensity of staining for a given region of the image. Optical density measures were normalized to minimize differences between replications of immunohistochemistry. First, a background measure-
ment was taken by placing a square outline, 4 times, on nonoverlapping, unstained areas of each section. The mean of these 4 measures provided the background optical density for each section. The ARC or PVN was outlined, and optical density of these regions was obtained. All mean background optical density measures were subtracted from the mean value for ARC or PVN staining.

Statistical Analyses

Proportions (%) entering torpor were analyzed with Chi-square or Fisher exact test. Correlations were determined with Pearson product moment. Torr_{max} (all animals) during 2DG testing was analyzed by one-way repeated measures ANOVA. All other comparisons were made with one- or two-way ANOVA (or ANOVA on ranks) with appropriate post hoc pairwise comparisons or by t-test (or rank sum test). Actual values for most statistical comparisons have been omitted for the sake of clarity. All comparisons were two-tailed and considered statistically significant if P < 0.05.

Procedures

Experiment 1: Photoperiod-dependent torpor and MSG ablation of arcuate nucleus. (Note: These animals participated in the 2DG experiment prior to being moved to the SP.) Adult Siberian hamsters postnatally treated with MSG (n = 33) or saline (n = 19) and previously acclimated to $T_{a}=10^\circ C$ were switched to a photoperiod with an SP (10:14-h light-dark cycle). Four days before the change in photoperiod (week 0) and every 1–2 wk thereafter, body mass and Fur Color Index were measured. Hamsters underwent an additional surgical procedure, if necessary, to replace a malfunctioning transmitter or an exhausted battery. Torr records were closely watched for the first appearance of spontaneous torpor, which typically appears after $\sim 12$ wk of SPs (23). Torpor bouts were analyzed for $T_{torr}$ and duration of torpor.

At week 8, the study was terminated. The hamsters were deeply anesthetized before the gonadal and retroperitoneal white adipose tissue depots were excised and weighed, and the hamsters were transcardially perfused for histological procedures. The experiment was conducted using two cohorts of hamsters (n = 14 and n = 38). Although $T_{torr}$ records are available for all 52, fat depot mass data are only available for the latter 38.

Experiment 2: Food-restriction-induced torpor and MSG ablations of arcuate nucleus. At weaning, saline-treated (n = 12) and MSG-treated (n = 22) pups were housed individually and moved to a room with an SP as before but $T_{a}=22^\circ C$. Fur Color Index was evaluated at this time (week 0) and every 2 wk until week 8 then every week thereafter. The hamsters were anesthetized and underwent a brief surgical procedure during week 9 to place a transmitter in the abdo-

men for recording $T_{b}$ as described above. At the end of 10-wk adaptation to SPs, the hamsters were moved to an environmental chamber with the same SP but $T_{a}=10^\circ C$.

At week 14 (4 wk in the cold), weekly food intake of all hamsters was determined (from week 14 to 15), and average daily food intake was calculated. All hamsters were provided the daily allotment of food, representing 70% of their previous average daily intake (30% reduction) beginning at week 16. If food restriction severely reduced body mass (body mass <25 g for females and body mass < 30 g for males), food-restriction was changed to 85% of ad libitum or discontinued. Food-restriction lasted 10–14 days.

At week 18, body mass and Fur Color Index were recorded. All hamsters were then given a lethal injection of pentobarbital sodium and when deeply anesthetized gonadal and retroperitoneal fat depots were excised and weighed before the hamsters were transcardially perfused for immunohistochemical verification of MSG ablations. $T_{torr}$ data were analyzed with particular emphasis on week 16–18.

Experiment 3: 2DG-induced hypothemia and MSG ablation of arcuate nucleus. At $\sim 30$ days of age, the saline- and MSG-treated hamsters subsequently used in experiment 1 underwent a brief surgery to implant radiotransmitters while under ketamine anesthesia. After recovery from surgery, the hamsters were housed in an environmental chamber with an identical LP but $T_{a}=10^\circ C$.

After 3 wk acclimation to $T_{a}=10^\circ C$, the MSG-treated (n = 37) and saline-treated (n = 21) hamsters were each randomly divided into two groups. During the first week, one group was tested with 2DG, and the other group with sterile saline vehicle; treatments were reversed in the 2nd week for a counterbalanced design in which each hamster receives each treatment. Torr records were examined for the presence or absence of torporlike hypothemia. Because male and female Siberian hamsters respond comparably to 2DG in LPs (10), their data were combined for analysis.

RESULTS

Histology

MSG ablations were verified by NPY immunohistochemistry in all MSG-treated Siberian hamsters entering torpor (n = 15), a randomly selected comparable number of MSG-treated hamsters never entering torpor (n = 13), and a random sample of saline-treated hamsters (n = 7). Brain sections evidencing NPY-positive fibers and cells in the ARC (Fig. 1A) and fibers in the PVN (Fig. 1D) are shown in a representative saline-treated control hamster, an MSG-treated hamster expressing torpor (Fig. 1, B and E), and an MSG-treated hamster never

![Fig. 1. Photomicrographs of brain sections demonstrating labeled NPY fibers and cell bodies in the Arc and NPY fibers in the PVN of a representative saline-treated hamster (A and D, respectively), a representative MSG-treated hamster expressing torpor (B and E, respectively), and a representative MSG-treated hamster never entering torpor (C and F, respectively). Scale bar = 100 μm. (Arc, arcuate nucleus of the hypothalamus; PVN, paraventricular nucleus of the hypothalamus; VMH, ventromedial nucleus of the hypothalamus; V3, 3rd ventricle.)](http://ajpregu.physiology.org/.../R257)
entering torpor (Fig. 1, C and F). There was a ~22% diminution of NPY-positive cells and fibers in the ARC of MSG-treated vs. saline-treated hamsters, as evidenced by densitometry measurements (Fig. 2A; \( P < 0.05 \)). Within MSG-treated hamsters, there were no differences in NPY immunoreactivity between those hamsters entering torpor and those never entering torpor (Fig. 2A; \( P > 0.05 \)). Further, within MSG-treated hamsters entering torpor, there was no correlation between density of surviving ARC NPY immunoreactivity and the frequency of torpor (\( r = -0.31, P > 0.05 \)). Similar outcomes were observed for NPY immunoreactivity in the PVN, a primary target of ARC NPY-ergic projections (Fig. 2B).

Experiment 1: photoperiod-dependent torpor and MSG ablations of the ARC. Eighteen weeks of SPs significantly increased Fur Color Index in both male and female hamsters \((P < 0.05, \text{for both; Fig. 3A})\). Although MSG treatment moderated the degree of color change in males \((P < 0.05)\), female hamsters did not differ in fur color responsiveness between postnatal treatments (Fig. 3A).

The proportion of hamsters undergoing torpor-like hypothermia was significantly reduced in MSG-treated Siberian hamsters vs. those postnatally treated with saline \((\chi^2 = 11.05, P < 0.05; \text{Fig. 4A})\). The incidence of photoperiod-dependent torpor in Siberian hamsters was more than 3 times more likely to occur in saline-treated (68%) than MSG-treated hamsters (18%). In addition, among only those hamsters entering torpor and with uninterrupted data during the 6-wk observation period, \(T_{\text{b}}\) min during torpor was significantly lower in saline-treated than MSG-treated hamsters \((P < 0.05; \text{Table 1})\). Torpor duration was correspondingly twice as long in saline- vs. MSG-treated hamsters \((P < 0.05)\), but torpor frequency did not differ statistically between groups (Table 1).

Fig. 2. A: comparison of densitometry measurements in the arcuate nucleus of NPY fiber/cell immunoreactivity between Siberian hamsters treated postnatally with saline vs. MSG, and a comparison within MSG-treated hamsters of the NPY immunoreactivity of those hamsters exhibiting torpor (Torpid) and those never entering torpor (Non-Torpid). B: comparison of densitometry measurements of NPY fiber immunoreactivity in the hypothalamic paraventricular nucleus after postnatal saline vs. MSG treatment, and within the MSG treatment group, a contrast of the NPY immunoreactivity of hamsters expressing torpor (Torpid) and those remaining euthermic (Non-Torpid). *Significantly different from saline-treated group.

Fig. 3. A: both MSG- and saline-treated Siberian hamsters demonstrate significant increases in Fur Color Index characteristic of exposure to SPs. B: longitudinal changes in body mass of the groups during 18 wk of SPs. Although there was a significant effect of sex (male vs. female) within each treatment over time, there was not a significant effect of treatment (saline vs. MSG) within either sex over time.
There was a significant main effect of sex (male vs. female) for body mass, gonadal fat mass, and retroperitoneal fat mass (ANOVA, $P < 0.05$, for all; Fig. 5, A–C). Neither body mass nor gonadal fat mass evidenced a significant main effect of treatment (ANOVA).

Table 1. Torpor characteristics of Siberian hamsters postnatally treated with saline vs. MSG and entering photoperiod-dependent torpor between Week 12 and Week 18 of SPs

<table>
<thead>
<tr>
<th>Postnatal Treatment</th>
<th>n</th>
<th>Mean # of Torpor Bouts</th>
<th>Torpid $T_b$, °C</th>
<th>Duration of Torpor, min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>11</td>
<td>11.3±3.0</td>
<td>21.3±0.4</td>
<td>342.7±24.1</td>
</tr>
<tr>
<td>MSG</td>
<td>5</td>
<td>6.0±1.8</td>
<td>23.5±0.6*</td>
<td>170.1±15.1*</td>
</tr>
</tbody>
</table>

All values are expressed as means ± SE. *Statistically significant from saline-treated.

Fig. 4. A: a markedly greater proportion of saline-treated hamsters (68%) entered photoperiod-dependent torpor than MSG-ablated hamsters (18%). B: two weeks of food-restriction and consequent weight loss failed to normalize the frequency of photoperiod-dependent torpor in MSG-treated hamsters (41%) to that of saline-treated hamsters (92%). (*Significantly different from MSG-treated group).

Fig. 5. Within sex, there was no significant difference between MSG-treated vs. saline-treated Siberian hamsters in either body mass (A), gonadal fat mass (B), or retroperitoneal fat mass (C). *Significant main effect of treatment (ANOVA).
treatment (saline vs. MSG) \((P > 0.05, \text{ for both; Fig. 5, A and B})\). There was, however, a main effect of saline vs. MSG treatment on retroperitoneal fat mass \((P < 0.05; \text{ Fig. 5C})\). Despite the significant main effect, there was no effect of treatment on retroperitoneal fat mass within either sex.

**Experiment 2: food restriction-induced torpor and MSG ablations of arcuate nucleus.** Exposure to 15 wk of SPs significantly increased Fur Color Index of both male and female Siberian hamsters postnatally treated with saline or MSG \((P < 0.05, \text{ for both; Fig. 6})\). In neither sex did treatment (saline vs. MSG) affect fur color.

The proportion of hamsters entering torpor after 18 wk of SPs and 10–14 days of food restriction to 70% of ad libitum intake was significantly reduced in Siberian hamsters postnatally treated with MSG (Fisher’s exact test, \(P < 0.05\); Fig. 4B). Forty-one percent of MSG-treated hamsters underwent torpor during the interval of food restriction compared with 92% of saline-treated hamsters. Food restriction during SPs increased the proportion of both MSG- and saline-treated hamsters entering torpor compared with similarly treated groups exposed to 18 wk of SPs alone (18% vs. 68%, respectively; experiment 1). A few hamsters \((n = 9)\) were unable to complete the entire interval of food restriction because of excessive weight loss but in those hamsters with uninterrupted data throughout the 14 days of food-restriction, torpor frequency, \(T_{b \min}\) during torpor and duration of torpor did not differ between treatments \((P > 0.05, \text{ for all; Table 2})\).

Although there was the usual significant sex difference in body mass and gonadal fat mass in food restriction/SP Siberian hamsters postnatally treated with saline vs. MSG during the interval of food restriction.

### Table 2. Torpor characteristics of Siberian hamsters postnatally treated with saline vs. MSG and entering torpor during the interval of food restriction

<table>
<thead>
<tr>
<th>Postnatal Treatment</th>
<th>(n)</th>
<th>Mean No. of Torpor Bouts</th>
<th>Torpid (T_{b \min}), °C</th>
<th>Duration of Torpor, min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>4</td>
<td>2.8 ± 1.2</td>
<td>23.8 ± 1.4</td>
<td>137.3 ± 26.7</td>
</tr>
<tr>
<td>MSG</td>
<td>10</td>
<td>2.1 ± 0.3</td>
<td>25.1 ± 0.9</td>
<td>113.8 ± 15.0</td>
</tr>
</tbody>
</table>

All values are expressed as means ± SE. The data presented here exclude hamsters not completing the entire food-restriction interval.

**Fig. 6.** MSG- and saline-treated Siberian hamsters both significantly increase Fur Color Index during 15 wk of SP exposure in experiment 2.

**Fig. 7.** There were sex differences in body mass \((A)\) and gonadal fat mass \((B)\), but not in retroperitoneal fat mass \((C)\) in food-restricted/SP Siberian hamsters. Postnatal treatment (MSG vs. saline) failed to affect either overall body mass \((A)\) or gonadal \((B)\) or retroperitoneal \((C)\) fat depot mass in SP hamsters after 2 wk of food restriction.
hamsters \((P < 0.05; \text{Fig. 7, A and B})\), the typical sex difference in retroperitoneal fat mass was absent \((P > 0.05; \text{Fig. 7C})\). There was no treatment effect on body mass, gonadal fat mass, or retroperitoneal fat mass between MSG- and saline-treated food-restricted/SP hamsters (Fig. 7, A–C). In addition, there was a marked negative correlation between body mass and torpor occurrence in food-restricted saline-treated male hamsters \((r = -0.99, P < 0.05)\), but there was no correlation between body mass and torpor in male MSG-treated hamsters \((r = -0.37, P > 0.05)\). (An insufficient number of saline-treated females completed the entire interval of food-restriction for statistical comparisons.) This lack of correlation is evident in the \(T_b\) records of comparable low body mass (Fig. 8, A and B) and high body mass (Fig. 8, C and D) saline- vs. MSG-treated hamsters.

Experiment 3: 2DG-induced hypothermia and MSG ablations of arcuate nucleus. Postnatal MSG treatment significantly reduced the proportion of hamsters entering hypothermia after 2DG injections \((\chi^2 = 4.78, P < 0.05; \text{Fig. 9})\). The proportion of MSG-treated hamsters entering 2DG-induced torpor-like hypothermia (38\%) was nearly half that of saline-treated controls (72\%) (Fig. 9).

There was a statistically significant main effect (ANOVA) of 2DG vs. vehicle injections on \(T_b\) \(_{\text{min}}\): 2DG injections markedly decreased \(T_b\) with comparison to vehicle injections \((P < 0.05; \text{Table 3})\). In addition, there was a significant main effect of postnatal saline vs. MSG treatment \((P < 0.05; \text{Table 3})\), as well as a significant interaction effect between treatments \((P < 0.05)\). Among all hamsters, 2DG injections decreased \(T_b\) \(_{\text{min}}\) in both postnatal treatment groups (saline vs. MSG); nevertheless, \(T_b\) \(_{\text{min}}\) in saline-treated hamsters was significantly lower than in MSG-treated hamsters \((P < 0.05; \text{Table 3})\). Among only 2DG-injected hamsters entering torpor-like hypothermia, \(T_b\) \(_{\text{min}}\) was significantly higher in MSG than in saline-treated hamsters \((P < 0.05; \text{Table 3 and Fig. 10})\), but comparisons of torpor duration between treatments did not reach statistical significance \((P > 0.05; \text{Table 3 and Fig. 10})\).
Table 3. Minimum body temperature ($T_{\text{b min}}$) in all animals after vehicle vs. 2DG injections in Siberian hamsters postnatally treated with saline or MSG, and $T_{\text{b min}}$ and torpor duration in only those saline- and MSG-treated hamsters entering torpor after 2DG injections

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>$T_{\text{b min}}$, °C (All Animals)</th>
<th>$T_{\text{b min}}$, °C (Torpid Only)</th>
<th>Duration of Torpor, min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline-control*</td>
<td>21</td>
<td>35.5 ± 0.1</td>
<td>25.3 ± 1.6</td>
<td>193.3 ± 91.5</td>
</tr>
<tr>
<td>Vehicle*</td>
<td>22</td>
<td>27.9 ± 1.4</td>
<td>25.3 ± 1.6</td>
<td>193.3 ± 91.5</td>
</tr>
<tr>
<td>MSG-ablated</td>
<td>37</td>
<td>35.5 ± 0.1</td>
<td>29.4 ± 0.5</td>
<td>103.6 ± 16.9</td>
</tr>
</tbody>
</table>

All values are expressed as means ± SE. *Statistically significant main treatments (postnatal treatment and pharmacological treatment; two-way ANOVA). †Statistically different from vehicle control; ‡Statistically different from 2DG group in saline-controls.

DISCUSSION

Postnatal MSG treatments markedly reduced the occurrence of photoperiod-dependent torpor in Siberian hamsters. In those few MSG-treated hamsters expressing torpor, torpor was significantly shallower and of a shorter duration than in saline-treated hamsters. The reduced frequency of torpor in MSG-treated hamsters was not a consequence of increased adiposity because 2 wk of food restriction that reduced fat mass did not normalize torpor occurrence to that of saline-treated hamsters. MSG-treatments, thus, appear likely to compromise food restriction-induced torpor as well. MSG-induced destruction of ARC neurons is the most parsimonious explanation of the disruption of photoperiod-dependent and food-restriction-induced torpor in Siberian hamsters. A conclusion supported by the similar elimination of food deprivation-induced torpor in National Institutes of Health Swiss laboratory mice after comparable ARC ablations due to perinatal MSG treatments (24).

MSG treatments primarily target ARC neurons colocalizing NPY/agouti-related peptide (AgRP) or proopiomelanocortin (POMC)/cocaine and amphetamine-related transcript, both of which possess leptin receptors (9, 26, 31). MSG-induced ARC ablations are thus likely to eliminate the leptin receptors necessary for the permissive effects of chronically reduced leptin concentrations in photoperiod-dependent torpor. High serum concentrations of exogenous leptin inhibit the occurrence of torpor and photoperiod-dependent torpor only occurs if leptin concentrations are markedly reduced (<2.6 ng/ml) (19). Postnatal MSG treatments may be ablating both the neuroendocrine transducer translating reduced leptin feedback into a permissive signal and effector mechanisms, for example, ARC NPY-ergic pathways, mediating torpor onset. Although reduced leptin feedback may be permissive for seasonal onset of photoperiod-dependent torpor, it nevertheless remains unclear at this time what specifically triggers this exaggerated circadian $T_{\text{b}}$ minimum on one day and not on another. Low leptin concentrations may be necessary, but they are not sufficient for initiating photoperiod-dependent torpor; leptin concentrations are comparable on torpid and nontorpid days (19). It also is evidently unrelated to food availability because Siberian hamsters routinely undergo photoperiod-dependent torpor in the laboratory with surplus food in their cages (e.g., experiment 1).

Elimination of photoperiod-dependent torpor (and possibly food restriction-induced torpor as well) is most likely due to MSG-induced damage to the hypothalamic arcuate nucleus. As mentioned before, the ARC possesses several peptidergic mechanisms that could potentially alter thermoregulation, the most prominent being NPY and POMC, which have opposing actions. POMC is a precursor for α-MSH, which inhibits food intake and enhances energy expenditure by activating the melanocortin-4-receptor (2, 55). AgRP is an endogenous melanocortin-4-receptor antagonist, increasing food intake and decreasing BAT heat production (55). NPY is widely known as a powerful orexin, but it also reduces energy expenditure and, more importantly, $T_{\text{b}}$. In fact, intracerebroventricular NPY injections in homeothermic rats completely suppress sympathetic nerve input to BAT (17) and concomitantly decrease $T_{\text{b}}$ by 1–3°C (30). Comparable intracerebroventricular injections of NPY (or NPY Y1 receptor agonist) in heterothermic Siberian hamsters, on the other hand, decrease $T_{\text{b}}$ by as much as 20°C, inducing hypothermia resembling natural torpor (36, 37). The marked enhancement of food deprivation-induced hypothermia resembling torpid torpor was not a consequence of increased adiposity because 2 wk of food restriction that reduced fat mass did not normalize torpor occurrence to that of saline-treated hamsters. The reduced frequency of torpor in MSG-treated hamsters was not a consequence of increased adiposity because 2 wk of food restriction that reduced fat mass did not normalize torpor occurrence to that of saline-treated hamsters. MSG-treatments, thus, appear likely to compromise food restriction-induced torpor as well. MSG-induced destruction of ARC neurons is the most parsimonious explanation of the disruption of photoperiod-dependent and food-restriction-induced torpor in Siberian hamsters. A conclusion supported by the similar elimination of food deprivation-induced torpor in National Institutes of Health Swiss laboratory mice after comparable ARC ablations due to perinatal MSG treatments (24).

Fig. 10. 2DG-induced torpor-like hypothermia in an MSG-ablated Siberian hamster (A) and a saline-treated hamster (B) whose $T_{\text{b min}}$ are representative of each respective group’s mean during hypothermia.
torpor in mice by ghrelin is eliminated in NPY knockout mice, also suggesting a role for NPY in torpor (24). Although NPY mechanisms and ARC mechanisms may be involved in torpor initiation, a specific role for ARC NPY-ergic pathways is speculative at best.

Photoperiod-dependent torpor persisted in a small percentage of ARC-ablated MSG-treated Siberian hamsters. The most likely cause may be a small, but critical, population of surviving ARC neurons that mediate torpor. There was no difference in density measurements of ARC NPY cell/fiber immunoreactivity between MSG-treated hamsters never entering torpor and MSG-treated hamsters entering torpor, and there was also no correlation between torpor prevalence and density of NPY immunoreactivity within those MSG-treated hamsters becoming torpid. The problem with MSG ablations in Siberian hamsters, as detailed by Ebling et al. (16), is the fine line between maximizing ARC damage and minimizing pup mortality. The MSG dose and injection schedule developed by Ebling et al. (16) for Siberian hamsters produces extensive ARC damage but ~20% of ARC NPY-positive cells inevitably survive MSG treatment. In the present research, ARC NPY cell and fiber immunoreactivity of MSG-treated hamsters was similarly ~22% that of saline-treated hamsters (see Figs. 1 and 2).

The ARC has repeatedly been shown to be the most sensitive structure to the neurotoxic effects of MSG (e.g., Refs. 24 and 49); nevertheless, damage to other sites has been described, including the area postrema (AP) (49). Even though AP ablation is without effect on photoperiod-dependent torpor (1), we cannot definitively rule out extra-ARC damage elsewhere, possibly contributing to the effects of MSG treatments on torpor.

It might be argued that MSG treatments prevented photoperiod-dependent torpor because of the somewhat greater fat reserves that are typical of MSG-ablated animals (e.g., Refs. 32 and 51), thus elevating circulating leptin concentrations sufficiently to inhibit torpor. Even though ARC leptin receptors may be ablated, there are also leptin receptors in the caudal brain stem, including the AP, nucleus of the solitary tract (NTS), and the dorsal motor nucleus of the vagal nerve (25). Because ablations of the AP/NTS complex have no effect on expression of photoperiod-dependent torpor in Siberian hamsters, it is unlikely that adiposity-dependent leptin concentrations inhibit torpor in MSG-treated hamsters. In addition, food availability restricted to 70% of ad libitum (and reduced fat reserves) in the SP in the present research increased the frequency of torpor in both groups of food-restricted hamsters, but it did not increase the frequency of torpor in MSG-treated hamsters to that of those treated with saline. It is unlikely, therefore, that reduced torpor frequency after MSG treatment was due to increased adiposity.

While the effect of food restriction cannot be parsed from that of photoperiod, it is clear that food restriction increased torpor frequency in both treatment groups. The findings also indicate that MSG-induced ARC ablations failed to increase the frequency of food restriction-induced torpor to that observed in saline-treated hamsters. In addition, there was interestingly no correlation between body mass and torpor incidence in MSG-ablated hamsters. Perhaps, this is because MSG-induced ARC ablations destroy the primary leptin receptor necessary for body fat feedback (12, 24, 50), and an appropriate energetic challenge can initiate torpor regardless of body fat reserves. Torpor can also be induced by food restriction in LPs, and even though it demonstrates several mechanistic differences from photoperiod-induced torpor, body mass in LP ARC-intact hamsters must still be markedly decreased before torpor is triggered (40).

Daily torpor is triggered in many small mammals, especially mice, by food deprivation (complete unavailability of food) (see Refs. 29 and 34). Food deprivation can induce torpor in mice in 24 h or less (20, 24, 34). Postnatal MSG treatments eliminate food deprivation-induced torpor in laboratory mice (24). Whether similar MSG ablations prevent food restriction-induced torpor in Siberian hamsters cannot be determined conclusively from the present study. Hamster species appear to respond differently to food deprivation and food restriction than other small mammal species. For example, neither Siberian (3) nor Syrian hamsters (47) show compensatory increases in food intake after food-deprivation. Siberian hamsters, similarly, fail to enter torpor after up to 48 h of food deprivation (J. Dark and K. M. Pelz, unpublished observations). The idiosyncratic nature of the neural mechanisms underlying responses to food deprivation and food restriction in Siberian hamsters, especially with relation to torpor, remains enigmatic.

MSG ablations significantly decreased the frequency of 2DG-induced torpor-like hypothermia. This effect is most likely due to ablation of forebrain, and not hindbrain, mechanisms. Although the AP/NTS is necessary for 2DG-induced food intake in rats (39) and estrous behavior disruption in Syrian hamsters (44), 2DG-induced torpor-like hypothermia fails to stimulate Fos-like immunoreactivity in the AP/NTS (35) and AP/NTS ablation fails to prevent 2DG-induced torpor-like hypothermia (1). 2DG treatment, on the other hand, increases NPY mRNA and NPY turnover in the ARC of rats (46). The intermediate effect of MSG ablations on 2DG-induced torpor-like hypothermia may reflect torpor initiation based upon a wide base of neural systems to which ARC NPY pathways make a substantial contribution. 2DG-induced torpor-like hypothermia is a regulated decrease in preferred Tb (10, 53). Because both photoperiod-dependent and food restriction-induced torpor also alter Tb setpoint (21), it is likely that at some point they all share a common mechanism. This is unlikely, on the other hand, with regard to torpor initiation in each case. Photoperiod-dependent and food restriction-induced torpor is clearly mediated by peripheral energy shortages, whereas 2DG-induced torpor-like hypothermia may more likely represent severe central glucoprivation (e.g., Refs. 8, 18, 35).

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