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Review

Short photoperiod reverses obesity in Siberian hamsters via sympathetically induced lipolysis and Browning in adipose tissue

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ABSTRACT

Changes in photoperiod length are transduced into neuroendocrine signals by melatonin (MEL) secreted by the pineal gland triggering seasonally adaptive responses in many animal species. Siberian hamsters, transferred from a long-day 'summer-like' photoperiod (LD) to a short-day 'winter-like' photoperiod (SD), exhibit a naturally-occurring reversal in obesity. Photoperiod-induced changes in adiposity are mediated by the duration of MEL secretion and can be mimicked by exogenously administered MEL into animals housed in LD. Evidence suggests that MEL increases the sympathetic nervous system (SNS) drive to white adipose tissue (WAT). Here, we investigated whether MEL-driven seasonally adaptive losses in body fat are associated with WAT lipolysis and browning. Hamsters were subcutaneously administered vehicle (LD + VEH) or 0.4 mg/kg MEL (LD + MEL) daily for 10 weeks while animals housed in SD served as a positive control. MEL and SD exposure significantly decreased the retroperitoneal (RWAT), inguinal (IWAT), epididymal (EWAT) WAT, food intake and caused testicular regression compared with the LD + VEH group. MEL/SD induced lipolysis in the IWAT and EWAT, browning of the RWAT, IWAT, and EWAT, and increased UCP1 expression in the IBAT. Additionally, MEL/SD significantly increased the number of shared MEL receptor 1a and dopamine beta-hydroxylase-immunoreactive neurons in discrete brain sites, notably the paraventricular hypothalamic nucleus, dorsomedial hypothalamic nucleus, arcuate nucleus, locus coeruleus and dorsal motor nucleus of vagus. Collectively, these findings support our hypothesis that SD-exposed Siberian hamsters undergo adaptive decreases in body adiposity due to SNS-stimulated lipid mobilization and generalized WAT browning.

1. Introduction

Seasonally adaptive responses such as changes in adiposity are induced by seasonal changes in photoperiod length. Siberian hamsters (*Phodopus sungorus*) have proven to be an outstanding model with which to study these naturally-occurring changes in obesity. During exposure to a long-day 'summer-like' photoperiod (LD) hamsters become obese (i.e., 50% body fat) but display a naturally-occurring adaptive loss of ~30% body fat when transferred to a short-day 'winter-like' photoperiod (SD) [1,2]. These photoperiod-dependent effects are mediated by changes in the duration of melatonin (MEL) secreted by the pineal gland. The seasonal changes in lipid mass can be mimicked in the laboratory by manipulating the duration of circulating MEL. For example, exogenously administered MEL can be used to increase the duration of circulating MEL in hamsters housed in LD resulting in a loss of body fat mimicking that seen in SD (for review see: [3,4]). The MEL receptor subtype mediating the body fat and other photoperiodic responses is the MEL1a receptor (for review see: [4,5]).

Although MEL plays a critical role in regulating the seasonal rhythmicity of body weight, it does not stimulate lipolysis in vitro [6] indicating that other signals are responsible for triggering SD-induced loss of body fat. The sympathetic nervous system (SNS) innervation of white adipose tissue (WAT) is sufficient and necessary for the initiation of WAT lipolysis [7]. Importantly, we determined that MEL1a receptor mRNA is colocalized in neurons that comprise the central SNS outflow circuitry from the brain to WAT [8]. We also found that exposure to SD photoperiod stimulates the SNS drive to WAT as indicated by increases in norepinephrine turnover [9]. Taken together these data support the hypothesis that MEL activation of MEL1a on the central SNS efferent neurons to WAT triggers lipolysis and ultimately reverses the obese phenotype of LD animals by accelerating SNS drive on WAT.

It has been reported that MEL decreases body mass due to increases in energy expenditure in the BAT [10,11]. Another potential factor in seasonal obesity reversal is the ability to harness heat production by converting white adipocytes to a beige phenotype via specific increases in the SNS drive to these browned WAT depots (for review see: [12]).

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Evidence suggests that recruitment of classical brown adipocytes in WAT can be induced by various metabolic stimuli such as cold exposure or browning agents, as the result of β3-adrenoceptor (β3-AR) stimulation (for review see: [12,13]). This process involves induction of mitochondrial uncoupling protein 1 (UCP1), which uncouples oxidative phosphorylation from ATP synthesis, releasing chemical energy as heat. In turn, the expression of UCP1 is regulated by specific transcriptional factors, most notably peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α) ([14]; for review see: [15]). Both UCP1 and PGC-1α are found at high levels in multilocular brown adipocytes of the brown adipose tissue (BAT), thus serving as brown/beige fat-specific markers. In support of the SNS impact on WAT browning, Himms-Hagen and colleagues [16] demonstrated that chronic administration of the specific β3-AR agonist, CL316,243, triggered the multilocular phenotype of WAT adipocytes. Moreover, we previously reported that Siberian hamsters transferred from LD to SD in the laboratory without lowering ambient temperature exhibit increased β3-AR, PGC-1α and UCP1 mRNA expression in the retroperitoneal WAT (RWAT), the only fat pad examined thereat [17].

It has been shown that the dorsomedial hypothalamic nucleus (DMH), containing orexigenic neuropeptide Y (NPY), is critical in inducing browning phenotype of adipocytes specifically in the inguinal IWAT (IWAT) [18]. Selective knockdown of NPY in the DMH triggers IWAT browning and chemical IWAT SNS denervation blocks browning response [18] suggesting that browning effect is mediated by WAT SNS innervation. In this study, we tested the hypothesis that MEL-driven seasonally adaptive losses in body fat are due to SNS-stimulated lipolysis, browning of WAT and increased energy expenditure as a result of enhanced UCP1 expression in the BAT.

2. Methods

2.1. Animals and photoperiodic conditions

Adolescent male Siberian hamsters (*Phodopus sungorus*; 2 months old, total $n = 45$) from our breeding colony were single-housed in a long-day (LD) photoperiod (16 h:8 h light:dark cycle with lights on at 0300 Eastern Standard Time; at 22 ± 2 °C) with ad libitum access to water and regular chow (#5001; 3.4 kcal/g, protein – 29.8%, fat – 13.4%, carbohydrates – 56.7%; Ralston Purina, St. Louis, MO) for 2 weeks before they were randomly assigned to one of two photoperiodic conditions. One third of LD hamsters ($n = 15$) were transferred to SD photoperiodic condition (8 h:16 h light:dark cycle; at 22 ± 2 °C) while the remaining two-thirds of hamsters ($n = 30$) continued to be housed in LD. Half of the LD hamsters were given a single subcutaneous injection of ethanolic saline (1:9 parts; LD + VEH group) or MEL [16 µg in 0.15 ml ethanolic saline MEL (LD + MEL), prepared fresh daily from a stock solution of 500 µg/ml MEL in 100% ethanol] daily for 10 weeks at 3 h before lights out. SD animals served as a positive control. Body mass and food intake were measured at 0900 h weekly throughout the experiment as previously described [19]. All procedures were approved by the Georgia State University Institutional Animal Care and Use Committee and are in accordance with Public Health Service and United States Department of Agriculture guidelines.

2.2. Tissue sampling and Western blotting

After 10 weeks all animals were euthanized with pentobarbital sodium (300 mg/kg) and approximately 100 mg of each fat pad [the interscapular brown adipose tissue (IBAT), IWAT, RWAT, epididymal WAT (EWAT)] as well as testes were rapidly removed snap frozen in liquid nitrogen and stored at − 80 °C. The tissues were homogenized in cold homogenization buffer [50 mM HEPES, 100 mM NaCl, 10.0% SDS, 2 mM EDTA, 0.5 mM DTT, 1 mM benzamidine, protease inhibitor cocktail (Calbiochem, EMD Chemicals, Gibbstown, NJ), and phosphatase inhibitor cocktail (Thermo Fischer Scientific, Rockford, IL)] and then centrifuged at 13,000*g* for 10 min at 4 °C. The supernatants were measured for protein content using the bicinchoninic acid protein assay kit (Thermo Fisher Scientific, Rockford, IL). Samples containing 10 µg of protein were mixed with loading buffer, heated at 95 °C for 5 min, electrophoresed on a low-bis SDS-PAGE [10.0%:0.08% acrylamide:bis] and transferred to polyvinylidene difluoride membranes. Each membrane represented a single gel and was cut in half so that immunoblotting could be done on duplicate lanes with different antibodies. The membranes were reacted with primary rabbit anti-UCP-1 (1:1000; Abcam, Cambridge, MA), PGC-1α (1:1000; Novus Biologicals, Littleton, CO), hormone-sensitive lipase (HSL), phosphorylated HSL (pHSL) and β-actin (each 1:1000; Cell Signaling Technology, Danvers, MA) antibodies for 2 days at 4 °C followed by incubation with secondary goat anti-rabbit IgG HRP-linked antibody, 1:1000; Cell Signaling Technology, Danvers, MA) antibody for 2 h at room temperature. The immunoblots were rinsed and incubated with LumiGLO chemiluminescent kit (Cell Signaling Technology, Danvers, MA) to visualize bands. The IBAT depots from Siberian hamsters were used as a positive control for UCP-1 and PGC-1α Western blotting showing the resulting proteins of the expected size.

2.3. Histology

Following fat pad extraction, hamsters were transcardially perfused with 0.9% heparinized saline followed by 4.0% paraformaldehyde in 0.1 M PBS. The brains were collected, postfixed in the same fixative for 3–4 h and then transferred to a 30.0% sucrose solution in 0.1 M PBS with 0.1% sodium azide at 4 °C overnight until the brains were sectioned on a freezing stage sliding microtome at 30 µm. For the double-label fluorescent immunohistochemistry, free-floating brain sections were rinsed in 0.1 M PBS (2 × 15 min) and 0.1% sodium borohydride in 0.1 M PBS followed by 30 min incubation in a blocking solution of 10.0% normal horse serum (NHS) and 0.3% Triton X-100 in 0.1 M PBS. Sections were then incubated in the mixture of primary rabbit anti-MEL1a (1:200; NBP1-71113, Novus Biologicals, Littleton, CO) and mouse anti-dopamine beta-hydroxylase (DBH) (1:500; clone 4F10.2, EMD Millipore, Billerica, MA) antibodies containing 1.0% NHS and 0.3% Triton X-100 in 0.1 M PBS for 48 h at 4 °C. Following rinsing with 0.1 M PBS (3 × 15 min), sections were incubated in the mixture of the secondary donkey anti-rabbit Alexa 488 (1:500; Jackson Immunoresearch, West Grove, PA) and donkey anti-mouse Cy3 (1:500; Jackson Immunoresearch) antibodies in 0.1 M PBS overnight at room temperature. For immunohistochemical controls, the primary antibody was either omitted or preadsorbed with the immunizing recombinant peptide (H00004543-Q01, Novus Biologicals) overnight at 4 °C resulting in no immunoreactive (-ir) staining. Brain sections were mounted onto slides (Superfrost Plus) and coverslipped using ProLong Gold Antifade Reagent (Life Technologies, Grand Island, NY).

2.4. Quantitative and statistical analysis

Intensity of Western blot bands was quantified with ImageJ (US National Institutes of Health, Bethesda, MA). Data are presented as percentage values normalized to LD + VEH control.

Brain images were viewed and captured using 100 × and 200 × magnification with an Olympus DP73 imaging photomicroscope (Olympus, Tokyo, Japan) with appropriate filters for Alexa 488 and Cy3. The single-labeled MEL1a and DBH images were evaluated and overlaid with the aid of CellSens (Olympus) and the Adobe Photoshop CS5 (Adobe Systems, San Jose, CA) software. Singly-labeled MEL1- or DBH-ir and doubly-labeled MEL1a + DBH-ir neurons were counted in every sixth section using the manual tag feature of the Adobe Photoshop CS5 software thus eliminating the likelihood of counting the same neurons more than once. Absolute neuronal numbers in the brain were averaged across each examined region from all animals. A mouse brain atlas [20] was used to identify brain areas because no Siberian

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