Original article

MOLECULAR METABOLISM

Lipolysis sensation by white fat afferent nerves triggers brown fat thermogenesis

John T. Garretson [1,3,*], Laura A. Szymanski [2], Gary J. Schwartz [4], Bingzhong Xue [1,2,3], Vitaly Ryu [2,3], Timothy J. Bartness [1,2,3]

ABSTRACT

Objective: Metabolic challenges, such as a cold environment, stimulate sympathetic neural efferent activity to white adipose tissue (WAT) to drive lipolysis, thereby increasing the availability of free fatty acids as one source of fuel for brown adipose tissue (BAT) thermogenesis. WAT is also innervated by sensory nerve fibers that network to metabolic brain areas; moreover, activation of these afferents is reported to increase sympathetic nervous system outflow. However, the endogenous stimuli sufficient to drive WAT afferents during metabolic challenges as well as their functional relation to BAT thermogenesis remain unknown.

Method: We tested if local WAT lipolysis directly activates WAT afferent nerves, and then assessed whether this WAT sensory signal affected BAT thermogenesis in Siberian hamsters (*Phodopus sungorus*).

Results: 2-deoxyglucose, a sympathetic nervous system stimulant, caused β-adrenergic receptor dependent increases in inguinal WAT (IWAT) afferent neurophysiological activity. In addition, direct IWAT injections of the β_3-AR agonist CL316,243 dose-dependently increased: 1) phosphorylation of IWAT hormone sensitive lipase, an indicator of SNS-stimulated lipolysis, 2) expression of the neuronal activation marker c-Fos in dorsal root ganglion neurons receiving sensory input from IWAT, and 3) IWAT afferent neurophysiological activity, an increase blocked by antilipolytic agent 3,5-dimethylpyrazole. Finally, we demonstrated that IWAT afferent activation by lipolysis triggers interscapular BAT thermogenesis through a neural link between these two tissues.

Conclusions: These data suggest IWAT lipolysis activates local IWAT afferents triggering a neural circuit from WAT to BAT that acutely induces BAT thermogenesis.

Keywords Lipolysis; Adipose innervation; WAT sensory; BAT thermogenesis; Denervation

1. INTRODUCTION

Adipose tissue is connected to the CNS via afferent and efferent nerve fibers directly innervating each fat depot. While adipose efferents are entirely catecholaminergic [1], adipose afferents are primarily unmyelinated c-fibers—positive for substance p and calcitonin gene-related peptide (CGRP)—and receptive to a variety of stimuli [2]. Although the anatomical reality of WAT afferents was initially discovered by Fishman and Dark [3], the first functional role of neural afferents arising from white adipose tissue (WAT) was demonstrated by Niijima [4] by injecting the adiposity hormone leptin directly into WAT then measuring neurophysiological activity in decentralized (sensory) nerve fibers connected to the tissue. These results have been interpreted to suggest that local leptin, secreted by WAT in proportion to adipocyte size and number, is transduced as a neural signal proportional to the amount of stored energy [5]. However, WAT afferents also increase their neurophysiological activity in response to adenosine and bradykinin—indicating an active role of these afferents in blood pressure sensation [6]. Despite the variety of signals sensed by WAT afferents, each neurophysiolgically effective stimulus similarly increases sympathetic nervous system (SNS) drive to WAT and other tissues in a positive feedback WAT-brain-SNS reflex circuit termed the "adipose afferent reflex" (AAR) [6,7]. Indeed, our viral tracing studies expose the neuroanatomical framework for this AAR wherein WAT afferents are networked to WAT efferents throughout metabolically relevant CNS nuclei (*e.g.* intermediolateral nucleus of the spinal cord, nucleus of the solitary tract, periaqueductal gray, dorsomedial, lateral, arcuate and paraventricular hypothalamus) [8]. In addition, these studies reveal a high degree of neuroanatomical overlap between neuronal populations at multiple peripheral and central levels of the neuraxis including WAT sensory and brown adipose tissue (BAT) SNS innervation [9,10]. Thus, functional and neuroanatomical sensory-motor links between WAT and the SNS have been established [11,12]; however, the sensitivity of WAT afferents to local lipolytic activity of the tissue and the potential for these afferents to induce heat production in BAT have not been investigated.

Among all functions of WAT, its catabolism and subsequent efflux of stored energy is a rapid and necessary process for the release of fuels

[1]Neuroscience Institute, Georgia State University, Atlanta, GA 30303, USA [2]Department of Biology, Georgia State University, Atlanta, GA 30303, USA [3]Center for Obesity Reversal, Georgia State University, Atlanta, GA 30303, USA [4]Department of Medicine and Neuroscience, Albert Einstein College of Medicine, Bronx, NY 10461, USA

*Corresponding author. 50 Decatur Street SE, Biology Department, Atlanta, GA 30303, USA. Tel.: +1 770 883 5804 (Daytime). E-mail: johnny.garretson@gmail.com (J.T. Garretson).

Received June 7, 2016 • Revision received June 24, 2016 • Accepted June 26, 2016 • Available online 30 June 2016

http://dx.doi.org/10.1016/j.molmet.2016.06.013

when energy demand is high, and this process is evolutionarily conserved across species including non-mammals [13,14]. During an energetic challenge such as cold exposure, norepinephrine released by axon terminals of post ganglionic SNS neurons binds β_3-adrenorecptors (β_3-AR) present on adipocytes [15] initiating a lipolytic cascade that depends on phosphorylation of hormone sensitive lipase (HSL) and leads to the preferential release of long-chain free-fatty acids (FFAs) and glycerol as useable energy [16,17]. Peripheral injection of 2-deoxyglucose (2DG), a glucoprivic sympathomimetic agent, causes an SNS-dependent mobilization of triglycerides from WAT and increases WAT afferent activity within minutes [9]. This efflux of substrates from WAT occurs locally first, then is circulated throughout the body/brain and oxidized as fuel when glucose is not readily available/sufficient for energy demand [18] or converted to heat via uncoupled oxidative phosphorylation by BAT, a tissue similarly dependent on SNS innervation to trigger thermogenesis [42]. Thus, we hypothesize that WAT afferents activated by glucoprivation may be sensing locally released lipolytic products, thereby transducing lipolysis into rapid neural signals communicated to the CNS [9]. Such sensation of lipolysis by WAT afferents may induce BAT thermogenesis because 1) lipolysis provides substrates that are required for BAT thermogenesis [19,20] and 2) there is significant overlap in the central neuroanatomical representation of WAT afferent projections and neurons that project to BAT efferents [9,10]. To investigate these hypotheses, we first tested if WAT lipolysis and/or its products were sufficient to drive spinal afferent nerves receiving sensory input from subcutaneous inguinal WAT (IWAT), then we tested whether stimulation of lipolysis sensing IWAT afferents triggers interscapular BAT (IBAT) thermogenesis.

2. METHOD

2.1. Animals

Adult male Siberian hamsters (*Phodopus sungorus*; ~3–4 months old) from our breeding colony were individually housed in a long day photoperiod (16 h:8 h light:dark cycle; at 22 ± 2 °C) with free access to water and rodent chow. Siberian hamsters were used because of the expanding literature characterizing the anatomy of their neuro-adipose axis [13] and the apparent conservation of adipocyte catabolism among mammalian species [14]. 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. Neurophysiological recordings of IWAT afferent nerve activity

Siberian hamsters ($n = 13$, unilateral recordings; $n = 16$, bilateral recordings) were anesthetized with ketamine (100 mg/kg)/xylazine (10 mg/kg, i.p.). Following a 2 cm dorsal ventral incision, IWAT and connective tissue was resected and isolated from surrounding tissue, after which the nerves innervating right and/or left IWAT were exposed. Single nerve bundles from these WAT nerves were isolated and severed proximal to the electrode placement site to disconnect efferent fibers, and the afferent (distal) ends of decentralized nerves were placed on platinum-iridium (32-gauge) hook electrodes. A petroleum jelly/mineral oil mixture (1:1) was applied to the sites to completely surround the electrode/nerve connection, and warm mineral oil was pooled into the recording area to insulate electrical noise, prevent aqueous infusions from leaking to other tissue, secure the nerve on the electrode, and reduce drying of tissue. A stable anesthetic plane was maintained with supplemental ketamine (50 mg/kg, s.c.) by examining toe pinch and eye blink responses throughout the recordings.

For unilateral recordings, baseline neurophysiological spike activity in IWAT afferents was recorded for 5 min as reported [9], then hamsters received either propranolol (40 mg/kg, i.p.) or sterile saline vehicle (i.p.) and spikes were recorded for an additional 5 min. Finally, 2DG (500 mg/kg, s.c.) was administered and IWAT afferent activity was measured for an additional 10 min.

For bilateral neurophysiological recordings, decentralized nerve bundles from left and right IWAT were each placed on electrodes, then three sharp stainless steel needles connected with Silastic tubing to Hamilton microsyringes (Hamilton Company, Reno, NV) were inserted into left and right IWAT along the lateral-coronal plane spaced 4–5 mm apart at a depth of approximately 4–5 mm each (diagram of procedure, Figure 2A). Neurophysiological activity arising from right and left IWAT nerve bundles was measured for 10 min prior to and then in response to a 15 μl injection of the β3 adrenergic receptor agonist CL316,243 (CL, 0.2 ng/kg or 0.1 ng/kg, 3.0 μl/min for 5 min) into one IWAT, while saline vehicle was simultaneously injected into the contralateral IWAT pad, CL stimulation is commonly used to simulate SNS activity to adipose tissue and reliably induces lipolysis *in vivo* and *in vitro* [21,22]. A separate cohort of animals was pretreated with the antilipolytic drug 3,5-dimethylpyrazole (DMP, 12 mg/kg, i.p.) [23–25] 20 min before IWAT infusions of CL (0.2 ng/kg) and saline. Eicosopentanoic acid (EPA) and arachidonic acid (AA) are both highly mobilized from adipose tissue during stimulated-lipolysis at ratios to other mobilized FAs that appear to be conserved across species [26,27]. Thus, EPA and AA were used as representative FAs—of potentially many more FA species—that may signal acute and local lipolysis to WAT afferents. Doses for EPA[2.94 μM in 15 μl Tocrisolve, ~8.91 mg/kg] and AA[0.27 μM in 15 μl Tocrisolve, ~0.81 mg/kg] were calculated from studies that measured net output of lipolytic products from adipose fragments in rats [27,28]; accordingly, physiologically relevant levels of EPA and AA were injected into WAT to assess whether lipolytic products stimulate WAT afferents directly. Infusions of drug or vehicle were counterbalanced by side to control for possible side bias of IWAT afferent innervations.

Extracellular signals were amplified 10,000 times with a differential AC amplifier set to low pass filter 100 Hz and high pass filter 1000 Hz (Model 1700; A-M Systems, Sequim, WA). Analog signals were visualized on an oscilloscope (Model 2530, BK Precision, Yorba Linda, CA), audio analyzer (Model 74-30-1; FHC, Bowdoin, ME), and digitized through a Digidata data acquisition system (Model 1440a; Molecular Devices, Sunnyvale, CA) at a 20,000 Hz sampling rate. Recordings were captured with accompanying Clampex 10.3 software and analyzed for the number of spikes based on a voltage threshold two standard deviations above mean non-signal noise via Clampfit 10.3 data analysis software package. All counted waveforms were visually screened, and non-physiological noise detected with the set threshold was identified and removed from analysis. Percentage change from baseline nerve activity was calculated for 10 min bins for each nerve recording session, then the difference between IWAT afferent activities from CL injected pads and saline injected pads was calculated and used for statistical analysis.

2.3. Western blot analysis of lipolysis induced by CL

Using an identical infusion protocol to bilateral electrophysiology experiments, but without IWAT afferent dissection/dissociation from the CNS, CL (0.1 ng/kg or 0.2 ng/kg) was infused into one IWAT pad simultaneously with a saline vehicle was infused into the contralateral IWAT pad. Animals receiving a high dose of CL (0.2 ng/kg) were pretreated with either DMP (20 min prior, 12 mg/kg, i.p.) or saline vehicle, and animals receiving a lower dose of CL (0.1 ng/kg)

