Published in final edited form as:
Int J Obes (Lond). 2010 October ; 34(0 1): S36–S42. doi:10.1038/ijo.2010.182.

Sympathetic and sensory innervation of brown adipose tissue

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Abstract

The innervation of brown adipose tissue (BAT) by the sympathetic nervous system (SNS) is incontrovertible and, with its activation, functions as the principal, if not exclusive, stimulator of BAT thermogenesis. The parasympathetic innervation of BAT only appears in two minor BAT depots, but not in the major interscapular BAT (IBAT) depot. BAT thermogenesis is triggered by the release of norepinephrine from its sympathetic nerve terminals, stimulating β3-adrenoceptors that turns on a cascade of intracellular events ending in activation of uncoupling protein-1 (UCP-1). BAT also has sensory innervation that may function to monitor BAT lipolysis, a response necessary for activation of UCP-1 by fatty acids, or perhaps responding in a feedback manner to BAT temperature changes. The central sympathetic outflow circuits ultimately terminating in BAT have been revealed by injecting the retrograde viral transneuronal tract tracer, pseudorabies virus, into the tissue; moreover, there is a high degree of colocalization of melanocortin 4-receptor mRNA on these neurons across the neural axis. The necessary and sufficient central BAT SNS outflow sites that are activated by various thermogenic stimuli are not precisely known. In a chronic decerebration procedure, IBAT UCP-1 gene expression can be triggered by fourth ventricular injections of melanotan II, the melanocortin 3/4 receptor agonist, suggesting that there is sufficient hindbrain neural circuitry to generate thermogenic responses with this stimulation. The recent recognition of BAT in normal adult humans suggests a potential target for stimulation of energy expenditure by BAT to help mitigate increased body fat storage.

Keywords

sympathetic nervous system; brown fat; sensory nerves; thermogenesis; UCP-1

Introduction

Brown adipose tissue (BAT) was first identified by Gesner[1] in 1551 in European marmots *Muris alpines*, and almost 400 years later was termed the ‘hibernation gland’ by Sheldon.[2] We now know that, in addition to its function in the rewarming associated with the hypothermia of hibernation or torpor,[3–5] BAT is involved in other thermoregulatory

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Conflict of interest
The authors declare no conflict of interest.

processes such as nonshivering thermogenesis (for review see Griggio,[6] Heldmaier *et al.*[7] and Sell *et al.*[8]), diet-induced thermogenesis[9] and febrile responses.[10,11] In common to all of these thermal responses is the ability of BAT to generate heat through the uncoupling of oxidative phosphorylation from electron transport (for review see Rousset *et al.*[12]). With the advent of functional and fairly substantial depots of BAT in normal adult humans (for review see Nedergaard *et al.*[13]), the significance of BAT in energy balance has taken on greater significance than when its importance was largely thought to be confined to rodents. The function of this review is to briefly describe some known facts about the innervation of BAT, including its sympathetic nervous system (SNS) and sensory innervation, as well its scant and BAT depot-specific parasympathetic innervation. Therefore, we will only review those cellular/signaling/molecular events necessary to help understand the functions of the sympathetic and sensory innervation of BAT for its thermogenic responses (for a comprehensive review of most aspects of BAT, see Cannon and Nedergaard[14]).

Most of what we know about BAT function, both at the systems level and at the molecular level, derives from the study of the largest BAT depot in rodents, interscapular BAT (IBAT). Because of its size, accessibility and clear innervation, IBAT has been the primary depot studied in these animals. Other BAT depots exist, including the cervical, mediastinal, pericardial and perirenal depots.[15] As noted above, there was confusion and denial of the presence and function of BAT in normal adult humans, largely because humans do not possess IBAT[16–19] (for review see Nedergaard *et al.*[13]). The BAT depots present in humans were revealed inadvertently when adults were scanned for tumor metastasis using fluorodeoxyglucose positron emission tomography to identify this metabolically active cancerous tissue (for review see Nedergaard *et al.*[13]). The result of these imaging studies revealed that normal adult humans clearly have substantial amounts of BAT in several depots located mainly in the supraclavicular and neck regions, with additional depots in paravertebral, mediastinal, para-aortic and suprarenal areas,[16–19] but importantly, not in the interscapular area. Moreover, human BAT possesses uncoupling protein-1 (UCP-1), the defining characteristic of BAT.[16,18,20]

SNS innervation of BAT

There was some initial confusion about the SNS innervation of BAT. That is, using histofluorescence, Wirsen[21] concluded in 1964 that there was no direct sympathetic innervation of brown adipocytes as evidenced by the absence of catecholaminergic nerve fibers in the parenchymal space; instead, this was observed only for blood vessels. One year later, however, Wirsen[22] described catecholaminergic varicosities found in the parenchymal space and among blood vessels using the same methods. The presence of both SNS parenchymal and vasculature innervation was soon verified by electron microscopy.[23] These and other data (for review see Himms-Hagen,[24] Bartness *et al.*[25] and Bartness and Song[26]) demonstrate that the sympathetic innervation of BAT is incontrovertible.

What are the central origins of the SNS outflow from brain to BAT?

Although there was a plethora of data showing sympathetic innervation of IBAT at the level of the fat pad, the origins of the central SNS outflow circuits ultimately terminating in IBAT

fell to mere speculation, with inferences generated following CNS manipulations (lesions, stimulation) that altered BAT function (surrogates of changes in BAT thermogenesis such as changes in UCP-1 gene and protein content, cytochrome oxidase content, guanosine–diphosphate binding, norepinephrine turnover (NETO), a neurochemical measure of sympathetic drive and sympathetic electrophysiological nerve activity to BAT; for review see Trayhurn and Milner[27]). The central origins of the SNS outflow from brain to BAT were first shown using a transneuronal viral retrograde tract tracer, pseudorabies virus (PRV), by us,[28] and later confirmed by others using this methodology.[29,30] Our work[28,31,32] was conducted in laboratory Siberian hamsters (*Phodopus sungorus*) and, to a considerably lesser extent, in laboratory rats.[28] Siberian hamsters show robust cold-induced BAT responses and use BAT for rewarming from shallow daily torpor induced in the laboratory by cold exposure combined with short 'winter-like' photoperiods (for review see Bartness[33]). We also previously demonstrated central origins of the SNS outflow from the brain to white adipose tissue (WAT) in Siberian hamsters using PRV.[31,34,35] Therefore, we have conducted the vast majority of our neuroanatomical and functional work on the sympathetic and sensory innervation (see below) of BAT (and WAT) in this species.

What is the functional evidence for the sympathetic neural control of BAT thermogenesis?

One proven strategy to test the necessity of intact BAT SNS innervation is to denervate the tissue. This can be carried out by surgical denervation and, moreover, can be accomplished using the unilateral surgical denervation model. Specifically, because both the lobes of IBAT have unilateral postganglionic innervation by the SNS in laboratory rats,[36,37] mice[38] and Syrian[39,40] and Siberian hamsters[41] (but see Seydoux *et al.*[42] and Girardier and Seydoux[43]), one side of the IBAT is surgically denervated and its contralateral side is sham-denervated, yielding a within-animal control. Thus, the beauty of the unilateral denervation model is that, for each animal, all circulating and central (brain) factors, as well as the animal's nutritional status, genetics and behavior, are identical, with the exception that one lobe of the IBAT is denervated and the other is not (for review see Bartness and Song[26]). A drawback of this model is that surgical denervation not only severs the descending SNS innervation to the IBAT but also the ascending sensory innervation (see below) from IBAT to the brain. This notwithstanding, our knowledge of the physiological functions of BAT mediated by its SNS innervation has been primarily advanced by using this model (for review see Bartness and Song[26]).

Using the unilateral denervation model, cold-induced increases in guanosine–diphosphate binding (that is, the capacity of the proton conductance pathway[27]), UCP-1 concentration (that is, thermogenic capacity[27]), total cytochrome oxidase activity (approximately equivalent to mitochondrial mass[27]), blood flow, mitochondriogenesis and cristae density, thyroxine 5′-deiodinase activity (an enzyme responsible for *in situ* conversion of thyroxine to triiodothyronine, a hormone involved in thermogenesis[44]), glucose uptake, as well as utilization and transporter number,[45] are all blocked or greatly diminished in the denervated compared with the innervated pad (for review see Bartness *et al.*[25]). Many or most of these cold exposure/acclimation-induced BAT responses can be restored in animals with

denervated BAT or can be mimicked by exogenous administration of norepinephrine (for review see Bartness *et al.*[25]), the principal sympathetic postganglionic neurotransmitter of the SNS. Collectively, denervation of BAT in cold-exposed animals highlights the importance of its sympathetic innervation for thermogenic responses. An analogous dysfunction of BAT can also be seen when it is denervated and then the animals are subsequently challenged with overfeeding (see Rothwell and Stock[46]) or fever (see Benzi *et al.*[47] and Rothwell[48]), once again demonstrating the importance of its sympathetic innervation for thermogenic responses.

Does IBAT have parasympathetic nervous system innervation?

The presence of parasympathetic nervous system (PSNS) has, until recently, been merely inferred on the basis of nonselective PSNS treatments that inhibit IBAT thermogenesis, such as systemic injection of atropine,[49] the cholinergic receptor postganglionic blocker, or by subdiaphragmatic vagotomy.[50,51] Both procedures, however, produce widespread peripheral effects, making it difficult to interpret the meaning of these data. Moreover, IBAT has no acetylcholine,[49] as determined by bioassay, or acetyl-cholinesterase activity,[49] the enzyme responsible for acetylcholine degradation, as determined histochemically. Relatively recently, using an immunocytochemical approach, laboratory rat BAT was tested for the presence of vesicular acetylcholine transporter and vasoactive intestinal peptide immunoreactivity.[15,52] There was an absence of vesicular acetylcholine transporter and vasoactive intestinal peptide immunoreactivity in IBAT, as well as in the cervical and perirenal depots.[15] Mediastinal BAT[15] and pericardial BAT[52] were the only depots provided with putative parasympathetic perivascular and parenchymal cholinergic nerves, as indicated by vesicular acetylcholine transporter immunoreactivity. The absence of vasoactive intestinal peptide-positive nerves suggests that these putative parasympathetic nerves are unlikely to be purely cholinergic.[15] Thus, at most, it appears that there is PSNS innervation of two minor BAT depots, but not of IBAT and the remaining BAT depots.

What neurochemicals are involved in the central BAT sympathetic circuitry?

Because PRV is not very cytopathic, PRV-infected neurons continue to generate enzymes, neurotransmitters and receptors.[53] Therefore, the SNS outflow to BAT from the brain can be labeled by PRV injection into IBAT and combined with *in situ* hybridization or immunocytochemistry for the receptor of interest, thereby yielding a map of central BAT SNS outflow neurons that possess the receptors. Such maps can serve as guides for subsequent studies in which receptor agonists or antagonists can be site-specifically injected to modulate BAT thermogenesis. For example, because central melanocortin receptor agonism (primarily the melanocortin 4-receptor (MC4-R)) decreases body weight that cannot be accounted for by the concurrent decreases in food intake,[54] increases in energy expenditure must be occurring. This could involve increases in BAT thermogenesis through its sympathetic innervation, but such an interpretation rests on the demonstration of the neuroanatomical reality of MC4-Rs colocalized on the BAT SNS outflow neurons. Therefore, we combined PRV labeling of the SNS outflow to IBAT with *in situ* hybridization for MC4-R mRNA in Siberian hamsters.[31] We found a remarkable degree of

colocalization of PRV-immunoreactive neurons with MC4-R mRNA, such that, across the neuroaxis, ~40 to >70% of all PRV-immunoreactive neurons for each brain structure demonstrated this level of colocalization.[31] These data suggest a profound role of melanocortins in BAT thermogenesis. Indeed, a single microinjection of melanotan II (MTII; the MC3/4-R agonist) into the third ventricle increases IBAT NETO in Siberian hamsters and IBAT temperature.[55] Moreover, a single microinjection of MTII into the paraventricular nucleus of the hypothalamus (PVH) increases IBAT temperature in awake, freely moving Siberian hamsters implanted with telemetric temperature transponders under their IBAT.[31]

Collectively, these data add significant support to the view that central melanocortins are important in controlling IBAT thermogenesis through the SNS innervation of this tissue, likely through MC4-Rs, and demonstrate the power of this combined tract tracing/receptor approach to the study of BAT function. We have applied a similar approach to test the role of melanocortins in lipolysis of WAT with somewhat analogous results. Specifically, we demonstrated the neuroanatomical reality of MC4-R mRNA colocalized on WAT SNS outflow neurons[56] and showed that central MC4-R agonism increases the sympathetic drive to WAT (NETO[55]), resulting in an induction of intracellular markers of lipolysis in only the WAT pads showing increased NETO.[57]

Functional approaches testing for central sites controlling BAT function

Another approach to test for the role of central sites controlling BAT thermogenesis that often precedes the neuroanatomical approach outlined above is the injection of the neurotransmitter in question into the brain ventricular system to stimulate a wide range of circumventricular brain sites and measure IBAT responses suggestive of changes in thermogenesis. For example, before our neuroanatomical tests for colocalization of MC4-R gene expression by central BAT SNS outflow neurons, we tested whether melanocortin receptor agonism of caudal hindbrain circuits would increase IBAT thermogenesis through its SNS innervation.[58] In this research we used the chronic decerebration model developed and championed by Harvey Grill and associates (for review see Grill[59]), in which a cut is made to completely separate the brain coronally at the level of the mesodiencephalic junction in laboratory rats.[60,61] With this single, albeit drastic, lesion, the sufficiency of caudal hindbrain structures for a given function can be readily tested. For example, we exposed chronic decerebrate (CD) rats or their sham controls to a series of declining ambient temperatures and measured heart rate, locomotor activity, BAT and heart NETO and body temperature.[62] CD rats responded to the cold in a manner quite similar to that of their neurologically intact controls, at least during the first 2h of cold exposure, with both groups increasing sympathetic drive (NETO) to IBAT and heart, locomotor activity and heart rate, as well as defending their body temperature.[62] Some of these responses were not fully maintained by CD rats with longer cold durations compared with their sham CD counterparts.[62] Such findings suggest that current models of body temperature control, and specifically BAT thermogenesis centered on the forebrain,[63] need further refinement to include peripheral temperature afferent information reaching the brainstem, as has been suggested to occur previously.[64,65] Relative to the sites of central melanocortin receptor agonism stimulating BAT thermogenesis, we first found that intraventricular injections of

MTII into the third or fourth ventricles equally increased our surrogate measure of IBAT thermogenesis, UCP-1 gene expression.[58] Furthermore, if IBAT is first denervated bilaterally, then fourth ventricular injections of MTII increase IBAT UCP-1 mRNA only in the sympathetically intact controls, and not in rats with surgically denervated IBAT.[58] Finally, CD rats given fourth ventricular injections of MTII increase IBAT UCP-1 mRNA statistically similarly to their neurologically intact controls, although some diminution of the response seems to be present.[58] Therefore, these data indicate a role for caudal brainstem melanocortin receptors in the control of energy expenditure, but do not discount potentially important hypothalamic and other forebrain contributions. Thus, the control of thermogenesis, as with other responses critical for survival, such as food intake,[59,66] seems to be a distributed system with multiple levels of control across the neuroaxis.[58]

Does BAT have sensory innervation?

BAT has marked sensory innervation at the level of the BAT depot as seen by immunohistochemical markers of sensory nerve-associated peptides that have been proven in other tissues (that is, substance P and calcitonin gene-related peptide). Specifically, there are substance P- and calcitonin gene-related peptide-immunoreactive nerve fibers within the parenchyma, as well as surrounding the vasculature of this BAT.[67–69] Attempts to understand the function of this sensory innervation has largely rested on the effects of global sensory denervation produced by systemic injections of capsaicin, the pungent part of red chili peppers that is a specific toxin for unmyelinated sensory nerves as well.[70] This manipulation results in general decreases in BAT growth (mass, protein content), mitochondrial content (cytochrome oxidase activity) and thermogenic capacity (UCP-1 content[71,72]), but because this sensory denervation is not BAT specific, these data are difficult, if not impossible to interpret. Studies in which BAT is specifically sensory denervated by direct injections of capsaicin, as we have carried out directly into WAT pads,[73,74] are ongoing for IBAT.

What are the central sensory circuits originating in BAT and projecting to the brain?

To define the central sensory circuits from IBAT to the brain, a different transneuronal virus was needed to travel anterogradely (following the flow of sensory nerve impulses in contrast to PRV, which travels opposite to the flow of the sympathetic nerve impulses). The H129 strain of herpes simplex virus-I fulfills this criterion.[75,76] This virus has been successfully used previously to label central gastric sensory circuits[77] and we recently used it to label WAT central sensory circuits.[78] In an ongoing study, we have seen extensive BAT sensory-labeled neurons in the brainstem, including the rostroventrolateral medulla, raphe magnus, raphe obscurus, nucleus of the solitary tract and, particularly noteworthy, the raphe pallidus, an area claimed to have preferential SNS efferents to BAT[79] (Vaughan, Song and Bartness, unpublished observations), although SNS outflow neurons are also labeled in the raphe pallidus after PRV injections into WAT.[78] The midbrain has considerably fewer infected cells, and those that occur primarily are in the ventral portions of the periaqueductal gray. Finally, considerable numbers of forebrain neurons are labeled with especially dense labeling in the PVH, subzona incerta and lateral and dorsomedial hypothalamus (Vaughan,

Song and Bartness, unpublished observations). Most of the infected structures listed above, and others not listed, have been implicated in the control of thermogenesis and energy balance. Moreover, there is considerable overlap in the structures that comprise the sensory inputs from IBAT to the brain and those that are part of the SNS outflow from brain to IBAT.[28,31] This begs the question as to whether there are SNS-sensory feedback loops innervating BAT, that is, single neurons that are a part of the sympathetic outflow to BAT and that receive sensory input from BAT.[79]

BAT SNS-sensory feedback loops

We previously demonstrated WAT SNS-sensory feedback loops by injecting PRV into WAT to label the SNS outflow from brain to WAT and, in the same fat pad (inguinal WAT), injected the H129 strain of herpes simplex virus-I.[80] Using that same strategy, we found single neurons in several brain areas that were dually infected in an ongoing study[80] (Song and Bartness, unpublished observations). That is, these neurons were infected with PRV and with H129, showing that they are part of the SNS outflow to IBAT and that they receive sensory inflow from IBAT (Song and Bartness, unpublished observations). This finding, albeit preliminary, begs another question: What is the function of the BAT SNS-sensory feedback loops? We do not know the answer to this question at this time; however, we speculate that these BAT SNS-sensory feedback loops could function to monitor one or both of two BAT events. First, these feedback loops could be involved in sensing the thermal status of IBAT analogously to heat-sensitive afferents that exist in other peripheral tissues.[81,82] Therefore, they could relay this thermal information to brain SNS outflow neurons involved in modulating the sympathetic drive to BAT and thus BAT thermogenesis. We have no data, however, to support this notion. The other possibility is that these feedback loops monitor BAT lipolysis. BAT contains multiple lipid droplets (thus its 'multilocular' lipid droplet phenotype[83]). It has been postulated that norepinephrine-induced lipolysis of BAT triacylglycerol, which composes the droplets, results in the release of fatty acids that activate UCP-1 to initiate heat production and that can also be oxidized by BAT to fuel this thermogenesis.[84] Key to the hydrolysis of triacylglycerol is the phosphorylation by protein kinase A, a cyclic AMP-activated protein kinase, of two proteins critical for lipolysis. Specifically, protein kinase A phosphorylates perilipin A and hormone-sensitive lipase. Perilipin A is thought to protect lipid droplets from lipolysis by coating them, but when phosphorylated, moves aside and/or functions as a scaffold for phosporylation of hormone-sensitive lipase that begins cleaving fatty acids from glycerol (for review see Brasaemle[85]). In the absence of protein kinase A phosphorylation of perilipin A, norepinephrine-induced activation of UCP-1, and thus thermogenesis, is blocked.[86] We have shown previously that sensory nerves innervating WAT increase their electrophysiological activity when the sympathetic drive to WAT is increased because of glucoprivation,[78] implying that they are sensitive to a lipolysis-associated event. Thus, the SNS-sensory feedback loops found in several brain areas for WAT and BAT could both serve to help control lipolysis of these tissues.

In summary, it is clear the BAT is innervated by SNS and sensory nerves, but only two minor BAT pads have PSNS innervation. BAT sympathetic denervation blocks thermogenic responses. Central BAT SNS outflow neurons possess high colocalization MC4-Rs and

MC4-R agonists injected to increase IBAT temperature. The caudal brainstem seems to be sufficient to trigger MC4-R agonism increases in IBAT thermogenesis, as well as being sufficient for acute BAT and other thermal responses to cold exposure. BAT SNS-sensory feedback loops seem to be a neuroanatomical reality, the function of which is unknown presently, but may serve to monitor the thermal activity of BAT and/or BAT lipolysis. Clearly the presence of substantial BAT in humans[16–19] highlights the potential importance of this tissue for nonshivering thermogenesis, diet-induced thermogenesis and febrile responses and makes its selective activation a potential target for pharmacological treatments designed to increase energy expenditure with the hope of curbing lipid accumulation by WAT and/or effectively reversing unwanted weight gain and obesity.

Acknowledgments

This work was funded by National Institutes of Health R37 DK35254 to TJB and the National Science Foundation Center for Behavioral Neuroscience Viral Tract Tracing Core through the Science Technology Center Program of National Science Foundation under agreement no. IBN-987654.

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