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Brain–adipose tissue cross talk

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While investigating the reversible seasonal obesity of Siberian hamsters, direct sympathetic nervous system (SNS) postganglionic innervation of white adipose tissue (WAT) has been demonstrated using anterograde and retrograde tract tracers. The primary function of this innervation is lipid mobilization. The brain SNS outflow to WAT has been defined using the pseudorabies virus (PRV), a retrograde transneuronal tract tracer. These PRV-labelled SNS outflow neurons are extensively co-localized with melanocortin-4 receptor mRNA, which, combined with functional data, suggests their involvement in lipolysis. The SNS innervation of WAT also regulates fat cell number, as noradrenaline inhibits and WAT denervation stimulates fat cell proliferation *in vitro* and *in vivo* respectively. The sensory innervation of WAT has been demonstrated by retrograde tract tracing, electrophysiological recording and labelling of the sensory-associated neuropeptide calcitonin gene-related peptide in WAT. Local injections of the sensory nerve neurotoxin capsaicin into WAT selectively destroy this innervation. Just as surgical removal of WAT pads triggers compensatory increases in lipid accretion by non-excised WAT depots, capsaicin-induced sensory denervation triggers increases in lipid accretion of non-capsaicin-injected WAT depots, suggesting that these nerves convey information about body fat levels to the brain. Finally, parasympathetic nervous system innervation of WAT has been suggested, but the recent finding of no WAT immunoreactivity for the possible parasympathetic marker vesicular acetylcholine transporter (VAChT) argues against this claim. Collectively, these data suggest several roles for efferent and afferent neural innervation of WAT in body fat regulation.

Sympathetic nervous system: Sensory nerves: Parasympathetic nervous system: Pseudorabies virus: Lipolysis

A critical ability of all animals is to meet energy demands quickly with utilizable metabolic fuels. In mammals glycogen serves as a rapidly available, but extremely limited, carbohydrate energy source. Energy is predominately stored as lipid in white adipose tissue (WAT) in the form of triacylglycerol (Newsholme & Leech, 1983). Access to this energy in fat can be mediated by several means, principally by the sympathetic nervous system (SNS) innervation of WAT. The present review starts by discussing the SNS innervation of WAT and then considers its sensory innervation and possible parasympathetic nervous system (PSNS) innervation. As the title of the review suggests, the cross talk occurring between the brain and WAT, which use these innervations as conduits, will be highlighted. However, the provocative and interesting cross talk between WAT and the brain that occurs via humoral factors will not be discussed.

Adrenal medullary catecholamines, once thought to be the principal stimulators of lipid mobilization, do not play a major role in lipolysis

Traditionally, adrenal medullary secretion of catecholamines, primarily adrenaline, is thought to be the underlying means by which lipid mobilization is stimulated. Thus, signals indicating the need for lipid-derived energy activate brain sites that, in turn, increase the SNS outflow to the intermedial lateral horn of the spinal cord impinging on the sympathetic preganglionic neurons that reside there. These neurons project separately to adrenaline- and

Abbreviations: CGRP, calcitonin gene-related peptide; FCN, fat cell number; MC4-R, melanocortin-4 receptor subtype; MEL, melatonin; PSNS, parasympathetic nervous system; PRV, pseudorabies virus; SD, short 'winter-like' days; SNS, sympathetic nervous system; WAT, white adipose tissue.

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noradrenaline-synthesizing chromaffin cells in the adrenal gland (Hillarp & Hokfelt, 1953; Edwards *et al*. 1996), and when their neuronal activity increases, there is an increase in the release of adrenaline, and to a lesser extent noradrenaline, into the circulation. Circulating adrenaline, in turn, stimulates membrane-bound WAT adrenoceptors (for review, see Lafontan & Berlan, 1993). Stimulation of these β-adrenoceptors activates a cascade of lipolytic intracellular steps resulting in increases in lipolysis (the breakdown of triacylglycerols into glycerol and NEFA), thereby supplying the needed lipid-derived fuels for oxidation (Newsholme & Leech, 1983). Despite mounting functional and histological evidence to the contrary (for reviews, see Bartness & Bamshad, 1998; Bartness *et al*. 2001), the dogma that adrenaline is the principal stimulator of lipolysis persists. This view probably arises because of the robust lipolytic activity of adrenaline when added to isolated adipocytes *in vitro* (for example, see White & Engel, 1958; Rizack, 1961). Evidence contrary to the primacy of adrenaline in triggering lipolysis *in vitro* is the inability of adrenal demedullation to block lipid mobilization triggered by several physiological conditions *in vivo*, e.g. glucoprivation (Nishizawa & Bray, 1978), electrical stimulation of the medial hypothalamus (Takahashi & Shimazu, 1981) and short photoperiod exposure in Siberian hamsters (*Phodopus sungorus*; Demas & Bartness, 2001*b*). As described later (p. 54), it is now more commonly believed that the sympathetic innervation of WAT, through the release of its primary postganglionic neurotransmitter noradrenaline, is the principal initiator of lipolysis (for review, see Bartness & Bamshad, 1998; Bartness *et al*. 2001; Dodt *et al*. 2003).

Naturally-occurring seasonal decreases in Siberian hamster body fat levels have served as a useful model to study the role of the sympathetic nervous system innervation of white adipose tissue

Although the innervation of WAT by the SNS has received support both neuroanatomically and functionally for more than 100 years (Dogiel, 1898; for review, see Bartness & Bamshad, 1998), it has only been in the last decade that irrefutable neuroanatomical and functional data have been forthcoming. The scientific community turned a blind eye toward this notion, probably because of the vigorous lipolytic response elicited by adrenaline when added to isolated adipocytes *in vitro*, as discussed earlier, and because initially only SNS innervation of blood vessels was seen in WAT (Wirsen, 1964; Daniel & Derry, 1969; Ballantyne & Raftery, 1974). This latter conclusion was undoubtedly drawn because white adipocytes of *ad libitum*-fed animals are filled with a single large lipid droplet that pushes the cell membranes into tight contact with neighbouring adipocytes, obfuscating the parenchymal space. After fasting, however, the cells shrink, revealing catecholaminergic innervation of both the vasculature and white adipocytes using histofluorescence (Diculescu & Stoica, 1970; Ballard *et al*. 1974; Slavin & Ballard, 1978; Rebuffe-Scrive, 1991).

A contribution to these more compelling data has been made through the present authors' search for the mechanisms underlying the reversibility of seasonal (photoperiod-induced) obesity exhibited by Siberian hamsters (for reviews, see Bartness & Wade, 1985; Bartness *et al*. 2002). As many of the neuroanatomical and functional examples of WAT innervation contained herein are based on these findings with the Siberian hamster, this model, which drew the authors into the 'brain–adipose tissue cross talk', will be briefly described.

When housed in long 'summer-like' days Siberian hamsters show a marked seasonal obesity (40–50% body fat) that gradually and naturally develops across the first 1–2 months of life (for review, see Bartness & Wade, 1985). Most remarkably, this obesity is completely reversible, such that when they are exposed to short 'winter-like' days (SD) they voluntarily rapidly lose body fat, with no decrease in food intake during the period of most rapid body mass decrease (for example, see Wade & Bartness, 1984; Bartness *et al*. 1989). The day length (photoperiod) provides a reliable 'noise-free' environmental cue (Turek & Campbell, 1979) that is responsible for the seasonal changes in body fat and other annual responses (e.g. pelage colour change, reproductive status, thermogenic capabilities) in Siberian hamsters, as well as in other species showing photoperiod-driven seasonal cycles (for review, see Underwood & Goldman, 1987; Bartness & Goldman, 1989). This photic information is received by the retina and transmitted via a multi-synaptic pathway to the pineal gland, where the pinealocytes synthesize and secrete melatonin (MEL) only at night. Thus, changes in the duration of the nocturnal secretion of pineal MEL faithfully code the night length, thereby signalling the progression of the seasons (photoperiods). Pinealectomized hamsters given exogenous peripheral MEL infusions that mimic the natural peak duration of nocturnal MEL secretion trigger the photoperiodic responses, including the changes in body fat (Bartness & Goldman, 1988; Song & Bartness, 1996, 1998).

Initially a hormonal intermediary for the MEL-induced changes in body fat was sought; however, none of the hormones that change seasonally and that also directly or indirectly affect body fat (e.g. thyroxine, insulin, gonadal steroids) could account for the SD-induced decreases in lipid stores (for review, see Bartness & Fine, 1999; Bartness *et al*. 2002). Thus, the SNS was considered as a possible mediator for the photoperiod–MEL-induced changes in body fat.

There is neuroanatomical and functional evidence for the sympathetic nervous system innervation of white adipose tissue

The inability of adrenal demedullation to block lipid mobilization under several physiological conditions (see p. 54) led to the examination of the SNS innervation of WAT as a possible mediator of the SD-induced decrease in body fat shown by Siberian hamsters (Demas & Bartness, 2001*b*). The first direct neuroanatomical evidence for the sympathetic postganglionic neurons was provided through injections of a retrograde fluorescent tract tracer Fluoro-Gold into inguinal WAT or epididymal WAT. To demonstrate these connections bi-directionally, an anterograde fluorescent tract tracer DiI was also injected into the

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sympathetic chain (Youngstrom & Bartness, 1995). Fluorescently-labelled cell bodies were found in the sympathetic chain as a result of retrograde labelling of the postganglionic nerves and rings of fluorescence around individual adipocytes as a result of anterograde labelling (Youngstrom & Bartness, 1995). Moreover, the distribution of postganglionic neurons innervating these two fat pads was found to be quite distinct, providing a likely neuroanatomical basis for the SD-induced differential lipid mobilization across fat pads (for example, see Bartness *et al*. 1989; Bartness, 1995, 1996), as well as for fat pad-specific differences in lipolysis shown in other rodent species and in human subjects. These data, along with an anecdotal report of sympathetic nerves innervating both white adipocytes and blood vessels, as revealed by histofluorescence combined with confocal microscopy (Rebuffe-Scrive, 1991), support the view that white adipocytes are sympathetically innervated. This phenomenon is not species-specific because, in addition to the studies of the SNS innervation of Siberian hamster WAT, parallel experiments were conducted in laboratory rats yielding similar findings (Youngstrom & Bartness, 1995).

These earlier studies gave no indication of the origins of the SNS outflow from brain to WAT. Connections from specific brain nuclei to WAT were, up to that time, inferred from lesion or stimulation studies in which the target site was destroyed or chemically or electrically stimulated and resulting changes in WAT growth or physiology were ascribed to disruption or activation of the presumed SNS outflow connections to WAT (for review, see Bartness & Bamshad, 1998). Another credible alternative explanation for the effects of brain lesions or stimulation on WAT would be that these manipulations affect peripheral endocrine organ function (e.g. PSNS innervation of the pancreas affecting insulin or glucagon secretion) resulting in changes in hormonal secretion that, in turn, affect WAT growth and/or physiology. In order to determine the credibility of the *post hoc* explanations ascribing the effects of brain lesion or stimulation to alterations in the SNS outflow to WAT, a transneuronal tract tracer, the Bartha's K strain of the pseudorabies virus (PRV), was used. PRV had been developed by other researchers to trace entire circuits within the same animal and had been used successfully to show the SNS outflow to the adrenal gland (Strack *et al*. 1989) among other peripheral tissues. Neurotropic viruses, such as the PRV, bind specifically to presynaptic neural membranes, fuse with their axonal membrane and deliver the uncoated capsids inside the axon. The capsids are transported by retrograde motors (probably dynein) to the cell body, where they replicate (Enquist & Card, 2003). The virus only exits the infected neurons via their dendrites, only infecting neurons that are synaptically connected to these PRV-laden cells. This process results in the retrograde labelling of functional chains of hierarchically-connected neurons within an animal (Card *et al*. 1990; Strack & Loewy, 1990). Initially PRV was injected into the inguinal WAT and epididymal WAT of Siberian hamsters (Bamshad *et al*. 1998). Retrogradely-infected cells were identified using immunocytochemistry throughout the neural axis, including the spinal cord (intermediolateral cell group, central autonomic nucleus), the brainstem (nucleus of the solitary tract, A5 regions and the C1/rostroventrolateral, rostroventromedial and caudal raphe nuclei/areas), midbrain (periaqueductal gray) and forebrain (hypothalamic: arcuate nucleus, dorsal, lateral, paraventricular, suprachiasmatic, nuclei and medial preoptic area; non-hypothalamic: zona incerta, medial amygdala, septum and bed nucleus of the stria terminalis; Bamshad *et al*. 1998). Many of the virus-labelled brain sites comprising the sympathetic outflow to WAT had been correctly deduced as components of this circuit using lesion or stimulation approaches (for review, see Bartness & Bamshad, 1998), except for one glaring omission, the virtual lack of PRV-labelled neurons in the ventromedial hypothalamic nucleus, an area implicated in dozens of lesion or stimulation studies (for review, see Bartness & Bamshad, 1998). This misplaced focus on the ventromedial hypothalamic nucleus probably occurred because manipulations of the ventromedial hypothalamic nucleus secondarily affect paraventricular nuclei descending pathways that pass adjacent to the ventromedial hypothalamic nucleus on their way to their brainstem and spinal cord destinations (for example, see Gold, 1973; Gold *et al*. 1977; Luiten *et al*. 1985).

Regulators of sympathetic outflow

A multitude of virally-labelled structures across the neuroaxis appears following the PRV injections into WAT (Bamshad *et al*. 1998, 1999; Shi & Bartness, 2001; Song & Bartness, 2001) and, moreover, within each of these structures there are scores of PRV-infected neurons. It seems unlikely that all these neurons in all these structures are activated under all conditions of lipid mobilization (e.g. with fasting, cold exposure or exercise). Instead, it is hypothesized that subsets of these structures and/or subpopulations of neurons within each structure are activated by different lipolytic stimuli. This hypothesis cannot be tested using double labelling for c-fos (the early immediate gene product that serves as a marker of cell activation; Hoffman *et al*. 1993) in these PRV-labelled circuits because the virus itself induces c-fos during early neuronal infection (Ozaki *et al*. 1996; Weiss & Chowdhury, 1998). A different approach was therefore used in an attempt to determine which of the SNS outflow neurons to WAT are involved in the SD-induced increased lipid mobilization. To label these neurons, *in situ* hybridization for MEL receptor mRNA combined with PRV labelling of the SNS outflow to WAT was used (Song & Bartness, 2001). As MEL does not directly affect lipolysis of isolated WAT cells *in vitro* (Ng & Wong, 1986), it was reasoned that there should be MEL receptors located on SNS outflow neurons to WAT of Siberian hamsters. First the SNS outflow neurons to WAT were labelled using PRV injected into inguinal WAT, combined with *in situ* hybridization for the MEL_{1a} receptor, the subtype through which the photoperiod causes seasonal responses in this and other species (Song & Bartness, 2001). Double-labelled neurons were found in several brain regions, including the suprachiasmatic nuclei (Fig. 1), an area previously shown to be critical for reception of the photoperiod-encoded MEL

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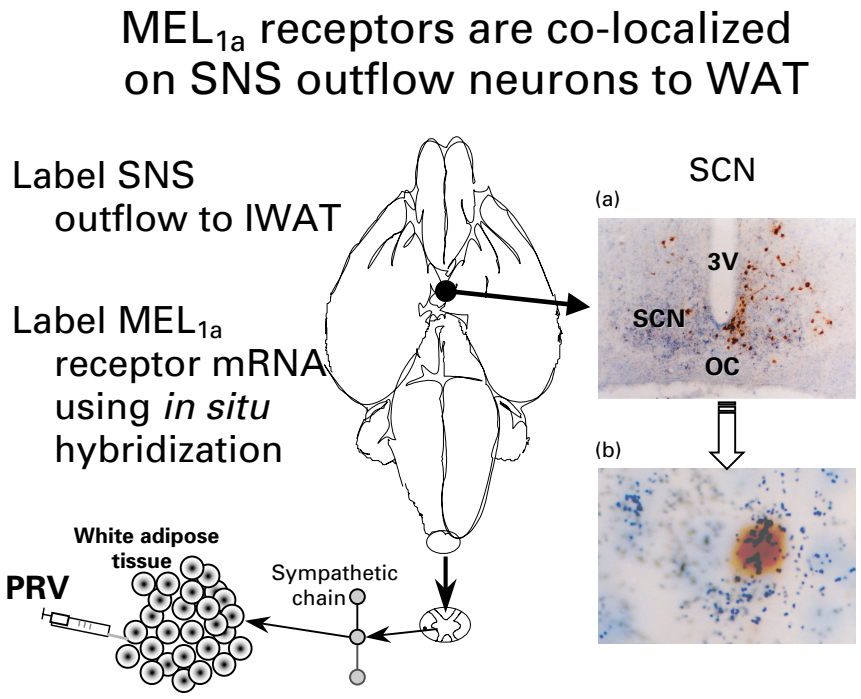

Fig. 1. Schematic representation of the double labelling of white adipose tissue (WAT) sympathetic nervous system (SNS) outflow neurons that also express mRNA for the functional melatonin receptor (MEL_{1a}) in Siberian hamsters (*Phodopus sungorus*) using the pseudorabies virus (PRV) and *in situ* hybridization respectively. The diagram is a coronal section of the brain at the level of the ventral hypothalamus showing double labelling of sympathetic outflow neurons to WAT with melanocortin-4 receptor (*MC4-R*) gene expression in the suprachiasmatic nuclei (SCN). (a) Brown staining indicates PRV labelling and the dark blue staining indicates gene expression for the MC4-R. (b) Enlarged photomicrograph shows one PRV-labelled neuron, as indicated by the brown staining, containing MC4-R mRNA, as indicated by the dark blue staining. 3V, third ventricle, OC, optic chiasm. (Adapted from Song & Bartness, 2001.)

signals that trigger SD responses (Bartness *et al.* 1991; Song & Bartness, 1998). Thus, the following scenario of SD-induced lipid mobilization has emerged. The increases in the duration of nocturnal MEL secretion resulting from the increases in night length cause increases in the stimulation of MEL_{1a} receptors in some of the neurons comprising the sympathetic outflow to WAT (e.g. suprachiasmatic nuclei). This, in turn, causes increases in the sympathetic drive to WAT (seen as an increase in noradrenaline turnover; Youngstrom & Bartness, 1995) thereby increasing lipolysis. Increased lipolysis, in turn, leads to decreases in WAT pad mass, reflected as decreased fat cell size (Bartness, 1996; Mauer & Bartness, 1996). To the authors' knowledge, this condition is the only one in which a stimulus that increases lipolysis can be traced from the environment to the adipocyte. Although the relevance for human obesity of photoperiod–MEL-induced changes in body fat is unknown, this model of naturally-occurring changes in body fat has proven useful in understanding the SNS neuroanatomy underlying lipid mobilization.

Using this powerful methodology for co-labelling central WAT SNS outflow neurons with expression of mRNA for their receptors, the possibility that melanocortin-4 receptor subtype (MC4-R) gene expression is co-localized with the brain sympathetic circuits innervating WAT has recently been tested. Although the melanocortins have been shown to markedly affect food intake and thermogenesis (for review, see Butler & Cone, 2002), their involvement in SNS-mediated lipolysis has been implied, but not directly tested. Chronic central administration of a synthetic MC4-R agonist melanotan-II (Haskell-Luevano *et al.* 1994) decreases WAT pad mass by approximately 50%, even when using pair-fed controls to account for its ability to decrease food intake (Raposinho *et al.* 2003). These results indicate that the melanotan-II-triggered decreases in body fat cannot be accounted for simply by its ability to decrease food intake. Furthermore, centrally-applied melanotan-II decreases the RQ, suggesting that lipid-derived fuels are being oxidized (Hwa *et al.* 2001). These and other data prompted the authors to test whether MC4-R mRNA is co-localized with PRV-labelled SNS outflow neurons to WAT in Siberian hamsters (CK Song, D Richard and T Bartness, unpublished results). Extensive co-localization of MC4-R mRNA with PRV-labelled SNS outflow neurons was found across the neural axis. All animals were found to have large numbers of PRV + MC4-R neurons in all PRV-labelled areas, including the paraventricular nuclei, preoptic area, bed nucleus of the stria terminalis and amygdala in the forebrain, periaqueductal gray in the midbrain and the nucleus of the solitary tract, lateral paragigantocellular

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nucleus, lateral reticular area, rostroventrolateral medulla and anterior gigantocellular nucleus in the brainstem, to name only a few of the more predominant co-localizations. This co-localization is the most extensive that has been seen in the work to date (Shi & Bartness, 2001; Song & Bartness, 2001) or in studies in the literature (for brief review, see Discussion in Shi & Bartness, 2001). These data suggest that MC4-R may play a prominent role in the modulation of SNS outflow neurons to WAT either through stimulation by the endogenous melanocortin agonist α-melanocyte-stimulating hormone and/or through inhibition by the naturally-occurring melanocortin-3 receptor and MC4-R antagonist agouti-related protein (Ollmann *et al*. 1997). Collectively, studies such as this one, and the co-localization of MEL_{1a} receptor mRNA with PRV-labelled WAT SNS outflow neurons (Song & Bartness, 2001), provide maps to guide future experiments for site-specific microinjections or implants designed to turn the WAT SNS outflow 'on' or 'off'.

The sympathetic nervous system innervation of white adipose tissue has at least three functions: lipolysis, the regulation of fat cell number and control of some white adipose tissue-secreted proteins

There are three recognized functions of the SNS innervation of WAT: (1) the principal initiator of lipolysis; (2) control of fat cell number; (3) control of some WAT-secreted proteins. The literature relating to the role of the SNS in lipolysis has recently been reviewed (Bartness & Bamshad, 1998; Bartness *et al*. 2001) and, therefore, will only be considered briefly. Functional studies have been conducted that take advantage of the unilateral innervation of pairs of WAT pads. One of a pair of WAT pads can be denervated with its contralateral counterpart serving as a within-animal neurally-intact control, thereby keeping circulating factors, age, nutritional status and the behavioural activity the same between fat pads. Surgically-denervated WAT shows a greatly diminished lipid mobilization compared with its neurally-intact controls across a variety of lipolytic stimuli, e.g. fasting (Clement, 1950; Cantu & Goodman, 1967; Bray & Nishizawa, 1978) and oestradiol treatment of ovariectomized animals (Lazzarini & Wade, 1991). Although local surgical denervation affords anatomical specificity compared with global sympathectomy using guanethidine (Powley *et al*. 1983) or 6-hydroxydopamine (Robidoux *et al*. 1995), it is not neuroanatomically selective because sympathetic and sensory nerves cannot be distinguished visually and therefore all types of nerves are severed. Indeed, surgical denervation decreases the immunoreactivity of tyrosine hydroxylase and calcitonin gene-related peptide (CGRP) -, indicating reduced sympathetic and sensory innervations respectively (Shi *et al*. 2005). A more selective approach than surgical denervation is chemical SNS denervation of WAT, which have been successfully accomplished using locally-injected guanethidine (Demas & Bartness, 2001*a*,*b*) and, more recently with greater reliability, using locally-injected 6-hydroxy-dopamine (R Bowers, CK Song, H Shi and T Bartness, unpublished results). Guanethidine-induced local SNS denervation of WAT severely blunts the SD-induced increase in lipid mobilization (Demas & Bartness, 2001*b*), as does surgical denervation (Youngstrom & Bartness, 1998), although only a complete blockade is achieved when adrenal demedullation is also added (Demas & Bartness, 2001*b*).

Recently, cross talk between white adipocytes and sympathetic neurons that influence lipolysis has been demonstrated cleverly and simply *in vitro* by co-culturing 3T3-L1 cells and rat superior cervical ganglia postganglionic sympathetic neurons (Turtzo *et al*. 2001). Characteristic morphology associated with each cell type, as well as cell type-specific markers, occurs in this situation, attesting to the authenticity of each cell type (Turtzo *et al*. 2001). Sympathetic neurons co-cultured with these adipocytes markedly inhibit β-adrenoceptor-stimulated lipolysis and leptin secretion (Turtzo *et al*. 2001). The effect on lipolysis is likely to be a result of increases in the release of neuropeptide Y by the co-cultured sympathetic neurons (Turtzo *et al*. 2001); neuropeptide Y is known to inhibit lipolysis *in vitro* (Castan *et al*. 1994) by stimulating membrane-bound adipocyte peptide YY receptors (i.e. neuropeptide Y receptors; Castan *et al*. 1993). The inhibition of WAT leptin release also may be initiated by stimulation of these receptors.

Sympathetic nervous system drive to white adipose tissue is not necessarily uniform across fat pads

How the SNS outflow is directed across sympathetic targets in general (for review, see Morrison, 2001; Sved *et al*. 2001), and across individual WAT pads specifically, is not well understood. It seems clear that the traditional theory proposed by Cannon (1939) of an 'all or nothing' activation of the SNS is inadequate to account for differential sympathetic drives across peripheral tissues. Thus, recent measures of SNS drive (noradrenaline turnover and electrophysiological activity of sympathetic nerves), as well as viral and non-viral tract tracing experiments, suggest that Cannon's (1939) hypothesis may represent the exception rather than the rule for activation of the SNS (for reviews, see Morrison, 2001; Sved *et al*. 2001). For example, the SNS control of blood flow to various vascular beds can occur independently of one another and of other organs (e.g. kidney and brown adipose tissue; Vague *et al*. 1980). In addition, in some conditions there are coordinated increases in sympathetic drives to two or more SNS target tissues, whereas in other conditions there are simultaneous increases to one of these targets and decreases to another. For example, with cold exposure noradrenaline turnover in brown adipose tissue (Young *et al*. 1982; Garofalo *et al*. 1996) and WAT (Garofalo *et al*. 1996) increases, but with fasting noradrenaline turnover in brown adipose tissue decreases and in WAT it increases (Migliorini *et al*. 1997). How the SNS drive to these two tissues types is regulated is a biological mystery. An additional puzzle is how the sympathetic drive across WAT pads (i.e. among inguinal WAT, epididymal WAT, retroperitoneal WAT etc.) is regulated. For example, noradrenaline turnover differs markedly across individual WAT pads of SD-exposed Siberian hamsters (Youngstrom & Bartness, 1995). Another example of differential

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sympathetic drive across WAT pads comes from a recent preliminary study (H Shi and T Bartness, unpublished results) in which acute glucoprivation was generated via injection of the glucose-utilization blocker 2-deoxy-D-glucose. In this case, 2-deoxy-D-glucose was found to markedly increase noradrenaline turnover in inguinal, retroperitoneal and dorsosubcutaneous WAT similarly, but does not alter epididymal WAT noradrenaline turnover (Fig. 2). These data suggest that acute glucoprivation causes differential sympathetic drive across WAT pads and further illustrates the phenomenon of the separate control of sympathetic drive across SNS target tissues. From a clinical perspective it is of paramount importance to determine the mechanisms underlying the differential mobilization of lipid from WAT, because the distribution of WAT is critical in relation to whether the secondary deleterious health consequences of obesity are manifested (Vague *et al*. 1980; Gasteyger & Tremblay, 2002). Moreover, even with modest decreases in visceral fat these secondary health consequences markedly improve (Pasanisi *et al*. 2001; Janssen *et al*. 2002), suggesting that an ability to selectively mobilize lipid from internal WAT depots would be of considerable clinical importance.

Sympathetic nervous system innervation of white adipose tissue also regulates fat cell proliferation

In addition to lipolysis the SNS innervation of WAT also influences WAT cellularity. It has long been recognized that WAT hypercellularity is a hallmark sign of obesity in both man and other animals (for reviews, see Kirtland & Gurr, 1979; Faust, 1984; Hausman *et al*. 2001). In addition, obesity typically is associated with decreases in SNS activity (for reviews, see Dulloo & Miller, 1987; Bray, 1990, 1991). It now appears that these two characteristics of obesity are related to one another; indeed, it may be hypothesized that decreases in SNS activity trigger hypercellularity. It appears that a high SNS drive to WAT inhibits, and a low SNS drive disinhibits (stimulates), fat cell proliferation, which was first demonstrated *in vitro*; noradrenaline added to white adipocyte precursor cells was found to inhibit their normal proliferation (Jones *et al*. 1992). This effect is blocked by pretreatment with the general β-adrenoceptor antagonist propranolol (Jones *et al*. 1992). Conversely, in Siberian hamsters (Youngstrom & Bartness, 1998), and in laboratory rats (Cousin *et al*. 1993), it has been demonstrated that surgical denervation of WAT produces pronounced (approximately 2-fold) increases in fat cell number (FCN) with little change in fat cell size *in vivo*. In addition, it has recently been reported that the magnitude of the denervation-induced increase in FCN varies between fat pads, with marked increases occurring in inguinal WAT but no change in epididymal WAT (Shi *et al*. 2004). This differential increase in FCN by surgical denervation may reflect the natural propensity for these pads to grow by hyperplasia (inguinal WAT) *v*. hypertrophy (epididymal WAT; DiGirolamo *et al*. 1998). Recent preliminary data suggest that these increases in FCN probably represent real increases in fat cell proliferation, rather than filling of existing pre-adipocytes. Specifically, surgical denervation increases the number of bromodeoxyuridine-labelled cells (i.e. dividing cells) that also are immunoreactive for AD3, a white adipocyte-specific membrane protein (Wright & Hausman, 1990; Kras *et al*. 1999), thus showing that they are proliferating white adipocytes (M Foster and T Bartness, unpublished results). Since surgical denervation severs all nerve types, it is possible that the lack of sensory innervation triggers increases in FCN. It has recently been found that WAT sensory denervation, accomplished by local injections of the sensory nerve neurotoxin capsaicin (Jansco *et al*. 1980; Ainsworth *et al*. 1981), and verified by decreased immunoreactivity for the sensory nerve-associated neuropeptide CGRP (Skofitsch & Jacobowitz, 1985), does not increase FCN (Shi *et al*. 2004). Thus, decreases in the SNS drive to WAT appear to be a critical stimulus for increasing fat cell proliferation or FCN.

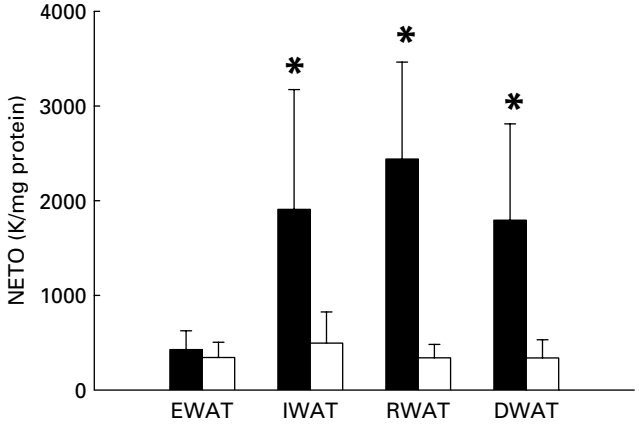

Fig. 2. Noradrenaline turnover (NETO) is differentially increased across white adipose tissue pads after glucoprivation induced by peripheral injections of 2-deoxy-D-glucose (2DG; ■) suggesting fat pad-specific control of sympathetic drive in Siberian hamsters (*Phodopus sungorus*). (□), Vehicle (saline; 9 g sodium chloride/l); IWAT, inguinal white adipose tissue; RWAT, retroperitoneal white adipose tissue; EWAT, epididymal white adipose tissue; DWAT, dorsosubcutaneous white adipose tissue. Values are means with their standard errors represented by vertical bars. Mean values were significantly different from those for the vehicle: * $P < 0{\cdot}05$. (H Shi and T Bartness, unpublished results.)

What is the mechanism underlying the denervation-induced increase in fat cell number?

If it were not for increases in FCN, then there would be an upper limit to the extent of adiposity reached by human subjects and other animals; however, hypercellularity is a classic sign of obesity (Faust, 1984). Despite the pivotal role played by fat cell proliferation in the obese state, little is known about the mechanisms underlying the process (for review, see Hausman *et al*. 2001), especially compared with the knowledge of factors that contribute to the process of fat cell differentiation. One obvious potential mechanism by which the SNS modulates FCN or proliferation is via the stimulation of β-adrenoceptors on adipocyte precursor cells. Evidence that noradrenaline prevents normal proliferation through β-adrenoceptors *in vitro* is the ability of the general β-adrenoceptor blocker propranolol to

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disinhibit the noradrenaline-induced inhibition (Jones *et al*. 1992), as discussed earlier. Another adrenoceptor subtype, the α_2-adrenoceptor, has also been implicated in adipocyte proliferation (Bouloumie *et al*. 1994; Valet *et al*. 1998). Specifically, increases in adipocyte α_2-adrenoceptor number precede the marked increase in FCN after surgical denervation of WAT (Cousin *et al*. 1993). The exact chain of events linking the increase in α_2-adrenoceptor number and stimulation of these receptors with the increase in fat cell proliferation is not known, but one possibility involves a compensatory increase in adrenal medullary catecholamine secretion that occurs after sympathetic nerve denervation (Takahashi *et al*. 1993). The steps beyond the stimulation of α-adrenoceptors resulting in this proliferation are not well understood, but locally released lysophosphatidic acid (Valet *et al*. 1998), a glycerophospholipid (Newsholme & Leech, 1983), may be involved. Agonists of the α_2-adrenoceptor trigger a rapid and prolonged release of lysophosphatidic in isolated WAT cells (Valet *et al*. 1998) and, moreover, lysophosphatidic acid added to preadipose cell lines triggers fat cell proliferation (Valet *et al*. 1998). Finally, the newly-discovered white adipocyte paracrine factor autotoxin (a type II ecto-nucleotide pyrophosphatase phosphodiesterase) may in turn stimulate proliferation via the release of lysophosphatidic acid (Ferry *et al*. 2003). Clearly, a deeper understanding of the mechanisms involved in fat cell proliferation in general, and the modulation of fat cell proliferation by the SNS specifically, requires further investigation.

Sympathetic nervous system outflow from brain can control the release of white adipose tissue-secreted peptides

Leptin is a cytokine that is synthesized and released primarily by white adipocytes (Maffei *et al*. 1995), and its discovery (Zhang *et al*. 1994) led to the notion that leptin informs the brain of body fat levels. This view, now considered dogmatic, is largely based on the frequent positive correlation between circulating leptin concentrations and the extent of adiposity (e.g. man (Considine *et al*. 1996; Dua *et al*. 1996; Ostlund *et al*. 1996) and laboratory mice (Frederich *et al*. 1995)); but leptin is also involved in other functions (e.g. reproduction, immunology; stress; for review, see Harris, 2000). There is an ever increasing number of exceptions to the positive correlation between body fat levels and circulating leptin concentration, suggesting that leptin might not be viewed as a perfect signal of adiposity.

The SNS outflow from the brain to WAT has been proposed as a principal controller of the secretion of leptin (Trayhurn *et al*. 1998). This view stems largely from the findings that conditions promoting increased SNS drive to WAT, such as cold exposure (Trayhurn *et al*. 1995) and fasting (Hardie *et al*. 1996), or direct stimulation of WAT β-adrenoceptors by receptor agonists (Mantzoros *et al*. 1996; Trayhurn *et al*. 1996) inhibit leptin gene expression and/or secretion. Conversely, disruption of the SNS drive to WAT via the catecholaminergic neurotoxin 6-hydroxy-dopamine or via α-methyl-*p*-tyrosine, a blocker of catecholamine (noradrenaline) synthesis, increases circulating leptin concentrations (Rayner *et al*. 1998; Sivitz *et al*. 1999). Collectively, these data support a primary role for the SNS innervation of WAT in leptin synthesis or release (increases in sympathetic drive decrease, and decreases in sympathetic drive increase, the synthesis or release of leptin).

Does white adipose tissue have parasympathetic nervous system innervation?

Recently, it has been reported that WAT has PSNS innervation (Kreier *et al*. 2002). This view is based largely on the presence of PRV-infected neurons in traditionally-accepted origins of PSNS premotor neurons, such as the dorsal vagal complex of the brainstem, after injections of the virus into WAT (Kreier *et al*. 2002). Such PRV-labelled neurons in this 'PSNS' area after injections into WAT have been reported (Bamshad *et al*. 1998), but they were attributed rogue SNS outflow neurons because the notion of wholly SNS or PSNS areas or nuclei in the brain has not withstood the test of time (for example, see Kalia *et al*. 1984; Gwyn *et al*. 1985). In addition, it is contended that WAT PSNS surgical denervation can be done selectively at the level of the WAT pad (Kreier *et al*. 2002). After this denervation, and in combination with a hyperinsulinaemic euglycaemic clamp, insulin-mediated glucose and NEFA uptake is reduced by approximately 30%, with hormone-sensitive lipase activity (involved in the hydrolysis of triacylglycerol) increased by approximately 50% (Kreier *et al*. 2002). Reductions in catabolic responses as a result of the presumed PSNS denervation of WAT suggest that the function of this parasympathetic innervation is to oppose the SNS catabolic actions in the tissue (Kreier *et al*. 2002), in much the same manner that these two innervations oppose one another in function in other tissues (e.g. heart). There is no corroborating neurochemical evidence to show that the phenotype of these neurons includes acetylcholine, the predominant PSNS postganglionic neurotransmitter, nor biochemical evidence for the acetylcholinesterase, an enzyme important in the degradation of acetylcholine (Ballantyne, 1968). Thus, the presence in WAT of vesicular acetylcholine transporter, a marker of PSNS innervation (for example, see Schafer *et al*. 1998), has recently been investigated (A Giradano, K Song, T Bartness and S Cinti, unpublished results). No vesicular acetylcholine transporter immunoreactivity has been found refuting possible PSNS innervation, as has been previously suggested (Kreier *et al*. 2002; for comparison, see Ballantyne, 1968; Bartness, 2002). Finally, preliminary studies have investigated the hypothesis that if first the SNS innervation of WAT is selectively eliminated and then the PRV injected, PSNS innervation should be preserved and infections of the PSNS outflow to WAT should be unabated. Thus, WAT was locally sympathetically denervated via injections of the catecholaminergic neurotoxin 6-hydroxy-dopamine, followed a few days later by injections of the PRV. In 6-hydroxy-dopamine-injected animals no PRV-infected cells were found anywhere in the brain, whereas the normal labelling of SNS outflow neurons by the virus was seen in hamsters injected with the 6-hydroxy-dopamine vehicle (A Giradano, K Song, T Bartness and S Cinti, unpublished results). Thus, these and other data discussed earlier raise some

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doubt as to the presence of PSNS innervation of WAT. Such innervation would be intriguing, however, and would afford WAT the neural control possessed by most other organs.

White adipose tissue also has sensory innervation that probably interacts with its sympathetic nervous system innervation

Sensory-motor innervation of tissues is the rule, not the exception; so it should not be surprising that there is sensory innervation of WAT (for reviews, see Bartness & Bamshad, 1998; Bartness *et al*. 2001). Sensory innervation of WAT was initially suggested by the identification of substance P in WAT (Fredholm, 1985). Substance P and CGRP are contained within, and released from, sensory neurons and thus are considered markers of sensory innervation (Hill *et al*. 1996). This initial biochemical analysis has been confirmed more recently using immunohistochemistry, showing that WAT contains CGRP- and substance P-immunoreactivity (Giordano *et al*. 1996). Direct evidence of the sensory innervation of WAT was first shown neuroanatomically when crystals of the fluorescent anterograde tract tracer True Blue were implanted in rat WAT, resulting in labelled bipolar sensory neurons in the dorsal root ganglia (Fishman & Dark, 1987). The functions of this sensory innervation of WAT are not well understood. One function involves the ability of leptin to increase peripheral SNS activity, as measured electrophysiologically; i.e. injections of leptin into one epididymal WAT pad increase sensory nerve firing rates from that epididymal WAT pad, while simultaneously increasing the sympathetic nerve activity of the contralateral epididymal WAT pad (Niijima, 1998). These data suggest that sensory nerves may possess leptin receptors, and that their activation could send afferent signals from WAT to the CNS via sensory nerves to trigger increases in the SNS outflow to WAT, thereby increasing lipolysis.

Another possible function of the WAT sensory innervation is its involvement in a negative feedback system that regulates lipid mobilization or accumulation through modification of the SNS drive to WAT. This view (Bartness & Bamshad, 1998) suggests interplay between sympathetic and sensory nerves to and from WAT respectively, and is based on evidence for such interactions in other tissues. For example, global sympathectomy or surgical denervation markedly increases CGRP immunoreactivity in the mesenteric and other vascular beds, as well as in most organs (Mione *et al*. 1992; Aberdeen *et al*. 1990). Conversely, a reciprocal response occurs after global sensory denervation, resulting in increases in sympathetic activity measured electrophysiologically (Ralevic *et al*. 1995).

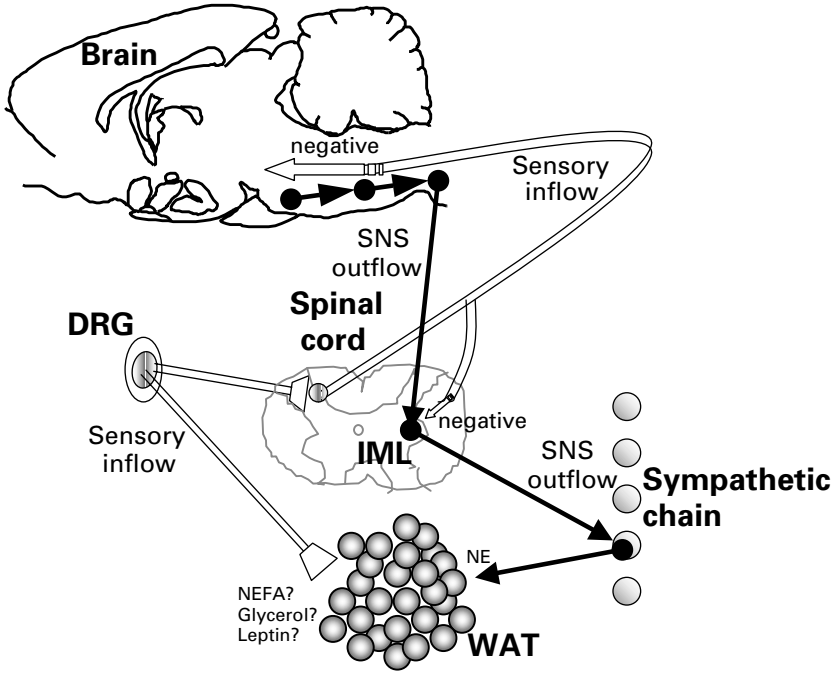

Fig. 3. Schematic diagram of hypothesized neural interplay between the sympathetic nervous system (SNS) outflow from brain to white adipose tissue (WAT). Sensory input (⇦) detects WAT substance indicative of the amount of lipid and/or its turnover, perhaps NEFA, glycerol or leptin via dorsal root ganglion (DRG) bipolar sensory neurons. Sensory information goes to spinal cord and then on to the brain to interact with the SNS outflow and/or a short-feedback loop may go to the intermedial lateral (IML) horn of the spinal cord; in either or both cases the sensory innervation performs as a negative feedback to the SNS innervation of WAT. The SNS outflow from brain to WAT (←) promotes lipolysis and inhibits fat cell proliferation via its principal postganglionic neurotransmitter, noradrenaline (NE). The sensory nerves may also or instead provide the brain with feedback about the lipid stores in WAT.

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These and other studies (for review, see Rubino *et al*. 1997) suggest cross talk between the sympathetic and sensory innervations of tissues and, based on these data, it is speculated that the sensory nerves innervating WAT may participate in a feedback loop to regulate the level of its sympathetic drive, thereby regulating lipolysis (Bartness & Bamshad, 1998).

Conclusions, speculations and future directions

WAT clearly has SNS and sensory innervation, with some data suggesting PSNS innervation (Kreier *et al*. 2002). As for the purported PSNS innervation of WAT, tract tracing showing innervation from dorsal vagal complex neurons to WAT that also express a PSNS neurochemical marker (e.g. acetylcholine, NO, vasoactive intestinal peptide) are needed, as well as functional studies such as electrical or chemical stimulation of these neurons to elicit responses from WAT that are the opposite of, or oppose, those of SNS activation.

Finally, the depth of the understanding of the function of the sensory afferent nerves emanating from WAT is certainly in its infancy. As noted earlier, it is believed that one of these mechanisms is to inform the brain of WAT pad lipid levels as local selective sensory denervation of WAT using capsaicin (H Shi and T Bartness, unpublished results) results in the reparation of the lipid deficit by lipid accretion in the other fat pads similar to that if the capsaicin-injected fat pads had been physically removed. Furthermore, anterograde trans-synaptic viral tract tracing is required to determine which areas of the brain receive this sensory innervation.

Collectively, the innervation of WAT by several types of neurons cannot be challenged, nor can its importance be underestimated. Additional roles for these innervations, especially for the control of the synthesis and release of WAT factors are extremely likely (e.g. leptin, adiponectin, TNF-α (Orban *et al*. 1999), which are important in lipolysis and fat cell proliferation. Understanding the roles of the sensory innervation of WAT and its interactions with the SNS innervation should also increase with a more detailed neuroanatomy of the sensory innervation. The differential activation of SNS outflow circuits to WAT and other energy-related sympathetic targets such as brown adipose tissue, as well as the differential SNS drives across WAT pads, should also prove enlightening. A schematic diagram of the cross talk between the SNS and sensory innervations of WAT is depicted in Fig. 3.

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