thematic review

Thematic review series: Adipocyte Biology

Sympathetic and sensory innervation of white adipose tissue

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Abstract During our study of the reversal of seasonal obesity in Siberian hamsters, we found an interaction between receptors for the pineal hormone melatonin and the sympathetic nervous system (SNS) outflow from brain to white adipose tissue (WAT). This ultimately led us and others to conclude that the SNS innervation of WAT is the primary initiator of lipid mobilization in these as well as other animals, including humans. There is strong neurochemical (norepinephrine turnover), neuroanatomical (viral tract tracing), and functional (sympathetic denervation-induced blockade of lipolysis) evidence for the role of the SNS in lipid mobilization. Recent findings suggest the presence of WAT sensory innervation based on strong neuroanatomical (viral tract tracing, immunohistochemical markers of sensory nerves) and suggestive functional (capsaicin sensory denervation-induced WAT growth) evidence, the latter implying a role in conveying adiposity information to the brain. By contrast, parasympathetic nervous system innervation of WAT is characterized by largely negative neuroanatomical evidence (viral tract tracing, immunohistochemical and biochemical markers of parasympathetic nerves). Functional evidence (intraneural stimulation and in situ microdialysis) for the role of the SNS innervation in lipid mobilization in human WAT is convincing, with some controversy regarding the level of sympathetic nerve activity in human obesity.— Bartness, T. J., and C. K. Song. **Sympathetic and sensory innervation of white adipose tissue. *J. Lipid Res.* 2007.** 48: **1655–1672.**

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Obesity is a disease of literally and figuratively enormous proportions (1, 2) and is an independent risk factor for type II diabetes, cardiovascular disease, stroke, and some cancers (3–5). Estimates of the mortality rate of overweight/obese individuals range from ~325,000 deaths per year (6) to as low as ~26,000 (7), making overweight/obesity either the number two or number seven cause of death in adults in the United States, respectively. Considerable research effort has focused on determining the factors involved in the development of obesity in nonhuman animals; consequently, our understanding of these is substantial, but still far from complete. Somewhat surprisingly, less effort has focused on determining the factors involved in the reversal of obesity in nonhuman animals. We have contributed to these latter efforts by studying the reversal of a naturally occurring seasonal obesity in Siberian hamsters (*Phodopus sungorus*). In the process of conducting this work, we discovered the importance of the sympathetic nervous system (SNS) in lipid mobilization from white adipose tissue (WAT) as well as more recently realizing the possible importance of the sensory innervation of WAT. Several overviews of the role of the SNS in lipid mobilization were published recently (8–11). To give a better understanding of how we came to the realization that lipid mobilization occurs primarily through the sympathetic innervation of WAT, we will describe some background studies focused on the reversal of photoperiod-induced obesity that ultimately led us to define and test the SNS innervation of WAT.

Abbreviations: BAT, brown adipose tissue; BrDU, bromodeoxyuridine; CGRP, calcitonin gene-related peptide; CNS, central nervous system; DRG, dorsal root ganglia; DWAT, dorsosubcutaneous white adipose tissue; EPI, epinephrine; EWAT, epididymal white adipose tissue; FCN, fat cell number; FCS, fat cell size; HIV, human immunodeficiency virus; HSL, hormone-sensitive lipase; HSV, herpes simplex virus; IBAT, interscapular brown adipose tissue; -ir, immunoreactivity; IWAT, inguinal white adipose tissue; LD, long day; MC4-R, melanocortin 4-receptor; MEL, melatonin; NE, norepinephrine; NETO, norepinephrine turnover; 6OHDA, 6-hydroxy-dopamine; PRV, pseudorabies virus; PSNS, parasympathetic nervous system; PVN, paraventricular nucleus; RWAT, retroperitoneal white adipose tissue; SCN, suprachiasmatic nucleus; SD, short day; SNS, sympathetic nervous system; TH, tyrosine hydroxylase; WAT, white adipose tissue.

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CHANGES IN THE PHOTOPERIOD (DAYLENGTH) TRIGGER A NATURALLY OCCURRING SUITE OF PHYSIOLOGICAL AND BEHAVIORAL RESPONSES

Animals living in temperate zones experience wide fluctuations in their environment, including changes in daylength, ambient temperature, rainfall, and vegetation. Therefore, it is not surprising that animals indigenous to such environments have evolved with the ability to markedly modify their physiology and behavior in anticipation of the approaching season. Through natural selection, animals that survived and reproduced were those responding not to the cues of the current season but to the cues occurring within the current season that predict the forthcoming season. This makes sense in that many of the seasonal physiological and behavioral modifications require weeks or months to be fully manifested and could not occur rapidly enough to be beneficial for the current season. For a wide variety of animals, including species of birds, lizards, amphibians, and mammals, the predominant environmental cue that triggers seasonally adaptive responses is the rate of change in the photoperiod: that is, how rapidly or how slowly daylength (or nightlength) lengthens or shortens (for reviews, see Refs. 12–16). If it was the absolute daylength, rather than the rate of progression from long to short or short to long photoperiods, that triggered seasonal responses, then it would be impossible to differentiate a spring day with 14 h of light from a fall day with 14 h of light.

When Siberian hamsters are exposed to long days (LDs), they develop a severe obesity [~50% body fat (17–19)], a level as extreme as that seen in genetically inbred strains of rats and mice [e.g., *ob/ob* mice (20) and Zucker *fa/fa* rats (21)]. The LD-induced obesity of Siberian hamsters is completely reversible by exposure to short days (SDs), with the decrease in body fat being most rapid during the first 5–6 weeks of SDs (17, 18, 22–24). Moreover, the body fat loss during this time occurs without a concomitant decrease in food intake but eventually (>6 weeks of SD exposure) is accompanied by an ~30% decrease in food intake (17, 25, 26); thus, the initial body fat loss is attributable to increased energy expenditure. Unlike other photoperiod-induced obesity models (27), the SD-induced decrease in WAT is nonuniform, with the more internally located visceral fat pads mobilizing their lipid stores first and to a greater extent than the more externally located subcutaneous fat pads (18, 28, 29). This differential lipid mobilization across WAT depots also is a characteristic of body fat loss in exercising/dieting/fasting humans (30, 31), although the exact pattern and fat pads involved are not homologous. Nevertheless, this highlights another advantage of using this model to study obesity reversal. It was this differential pattern of lipid mobilization and, as we shall see, differential sympathetic drive to WAT, that fascinated us early in our studies and prompted us to delve into the mechanisms underlying this phenomenon.

How does the daylength cue get transduced into a biological signal? The photoperiod cue is received by the retina and transmitted through a multisynaptic pathway that includes the suprachiasmatic nucleus [SCN; the primary biological clock (32)], the hypothalamic paraventricular nucleus (PVN), and the intermedial lateral horn of the spinal cord that ultimately terminates in the pineal gland (33), where this photic information is transduced into an endocrine signal via the rhythmic pattern of secretion of its principal hormone, melatonin (MEL) (15). Specifically, MEL is only synthesized and secreted by pinealocytes at night; thus, the duration of night is faithfully coded by the duration of nocturnal MEL secretion, thereby triggering seasonal responses: long durations of the circulating MEL signal short "winter-like" days (SDs), and short durations of circulating MEL signal long "summer-like" days (LDs) (for reviews, see Refs. 15, 34). The MEL receptor subtype mediating photoperiodic responses is the MEL_{1a} receptor [or the mt_1 receptor (35)]. MEL itself does not directly trigger lipolysis; incubation of isolated white adipocytes in vitro with physiological or even "industrial-strength" doses of MEL does not increase lipolysis (36). We tested hormones that both changed seasonally in Siberian hamsters and either directly or indirectly affected lipolysis in these or other animals (e.g., insulin, prolactin, glucocorticoids, gonadal steroids, thyroid hormones). None of these hormones, at least singly, accounted for the SD-induced decrease in body fat, as tested in depletion-repletion studies (for review, see Ref. 37).

THE SD (MEL)-INDUCED DECREASE IN BODY FAT IS VIA THE SNS INNERVATION OF WAT AND NOT THROUGH ADRENAL MEDULLARY EPINEPHRINE

Because of the largely negative nature of studies testing for the hormonal mediation of SD-induced decreases in adiposity in Siberian hamsters, we tested the role of adrenal medulla-secreted epinephrine (EPI). At that time, EPI was considered the principal initiator of lipolysis because of its ability to robustly simulate lipolysis (glycerol release) in vitro in isolated white adipocytes (38–41). Tests of this notion in vivo, however, do not support an important role of EPI in lipolysis. Specifically, despite adrenal demedullation removing the sole source of circulating EPI, lipid mobilization is unaffected by several stimuli known to stimulate lipolysis [e.g., glucoprivation (42) and electrical stimulation of the medial hypothalamus (43)], including SD-induced lipid mobilization in Siberian hamsters (44). Thus, EPI is not necessary for lipolysis.

Histological evidence for the SNS innervation of WAT has a long history beginning with Dogiel (45) in 1898, when he reported the staining of nerves innervating WAT. Confusion arose initially regarding which components of WAT received the SNS innervation, because there were descriptions of only the sympathetic innervation of blood vessels in WAT as well as other descriptions of the sympathetic innervation of the WAT parenchyma (for reviews, see Refs. 9, 11). It is now realized that the failures to detect neural fibers within the parenchymal space of WAT of

normal ad libitum-fed laboratory rats was the result of the tight packing of the fully loaded, lipid-filled adipocytes in the WAT matrix. By fasting animals and thus inducing lipolysis, the consequent lipid mobilization causes decreases in fat cell size (FCS), thereby exposing more parenchymal space and revealing catecholaminergic (via histofluorescence) innervation of both the vasculature and white adipocytes (46–49). The nerves innervating white adipocytes are not as boutons juxtaposed closely to the fat cells; rather, they are of the *en passant* variety (50).

This histofluorescence labeling of catecholaminergic nerve fibers within WAT is not considered direct evidence of SNS innervation; tract tracing of the sources of input to the WAT pads is the *sine qua non* of direct innervation of a tissue. Therefore, we used fluorescent tract tracers (DiI, FluoroGold) to demonstrate bidirectionally direct neuroanatomical projections of postganglionic neurons residing in the sympathetic chain to WAT (26). In addition, we found evidence of largely, but not completely, separate postganglionic neurons within the sympathetic chain innervating inguinal white adipose tissue (IWAT) and epididymal white adipose tissue (EWAT) (26). Such segregation of populations of postganglionic neurons innervating individual WAT pads could provide the neuroanatomical underlying basis for the differential mobilization of lipid across individual WAT depots, as occurs with most lipolysis-promoting stimuli [e.g., starvation (51, 52), estradiol (52), and leptin (53)]. A similar conclusion was drawn more than a decade later (54) when one of two strains of the pseudorabies virus (PRV), a transneuronal tract tracer (see directly below), each possessing a distinct fluorescent reporter, was injected into mesenteric and subcutaneous WAT. This resulted in the identification of an additional segregation of neurons innervating these two WAT pads but located a synapse earlier than those we found in the sympathetic chain (26), in the intermediolateral horn of the spinal cord [i.e., the sympathetic preganglionic neurons (54)]. Although partially satisfying, the determination of the preganglionic and postganglionic sympathetic innervation of WAT left open to speculation the origins of the sympathetic outflow to WAT from the brain.

CENTRAL NERVOUS SYSTEM ORIGINS OF THE SYMPATHETIC OUTFLOW TO WAT HAVE BEEN DEFINED USING VIRAL TRACT TRACING METHODOLOGIES

We were able to define the source of the premotor neurons participating in the sympathetic circuits innervating WAT because of the pioneering work of others using the Bartha's K strain of PRV to trace the SNS outflow to numerous peripheral tissues [e.g., adrenal (55) and pineal (33)]. In brief (for reviews, see Refs. 56, 57), neurotropic viruses, such as PRV, are taken up into neurons after binding to viral attachment proteins located on the surface of neuronal membranes and the viral envelope fusing with the cell membrane. The resulting capsids containing the viral core DNA enter the cytoplasm and, upon reaching the nucleus, hijack host machinery to produce its progeny, which are then exocytosed through the dendrites only. Neurons making synaptic contact with the infected cells then become exposed to relatively high concentrations of the new virions. The virions subsequently are taken up only at synaptic contact sites, and this process continues, causing an infection along the neuronal chain from the inoculation site in WAT to higher central nervous system (CNS) sites. The infected neurons can then be easily visualized using standard immunohistochemistry or, because PRV is relatively easily genetically engineered, isogenic versions of the virus have been constructed to produce fluorescent reporters [i.e., green (58) or red (59) fluorescent protein]. Because the transfer of the virus only is by a transynaptic mechanism, rather than by lateral spread to adjacent but unrelated neurons or by a nonsynaptic mechanism (60), the transynaptic transfer of the PRV after injection into WAT yields a hierarchical chain of functionally connected neurons from brain to WAT (for review, see Ref. 61).

We retrogradely labeled the SNS outflow from brain to WAT (IWAT and EWAT) in laboratory rats and Siberian hamsters (62) and later to retroperitoneal white adipose tissue (RWAT) in Siberian hamsters (63), thereby identifying CNS-SNS-WAT circuitries (for review, see Ref. 11, 64). The general patterns of infection after WAT inoculation with PRV prominently show that WAT (62), as well as brown adipose tissue (BAT) (65), receives input from CNS cell groups that are part of the general SNS outflow from the brain [PVN, A5 of the noradrenergic lateral tegmental system, caudal raphe region, rostral ventrolateral medulla, ventromedial medulla (66), and many other areas as well (67)]. Some of these include the hindbrain: area postrema, nucleus of the solitary tract, and raphe regions (e.g., pallidus, obscurus, magnus, and dorsal raphe and the raphe cap); midbrain: periaqueductal gray and pontine regions; and forebrain: hypothalamic arcuate, preoptic, SCN, PVN and dorsomedial nuclei, and thalamic paraventricular and reuniens nuclei (for a complete list, see Ref. 67).

Although there were some differences in the degrees of infection (i.e., neurons participating in the circuit) at several sites across the neural axis among these WAT pads in Siberian hamsters, the general patterns of infection were more similar than different for the same WAT depots between Siberian hamsters and laboratory rats (62, 63). This should not be misconstrued as a dismissal of "viscerotopic" sympathetic innervation of WAT at some point(s) across the neuroaxis (see above for evidence of preganglionic and postganglionic viscerotopic patterns of sympathetic nerves innervating WAT); however, unequivocal demonstration of the viscerotopic organization of WAT circuitries requires careful studies using isogenic strains of the PRV, each with unique fluorescent or other reporter injected into separate WAT pads (54). From the perspective of shared circuits, we found some common neurons in the sympathetic outflow circuits innervating a WAT (IWAT) and a BAT depot [interscapular brown adipose tissue (IBAT)] in a preliminary experiment (61). It would

not be surprising, therefore, to see some shared neurons at some level(s) of the neuroaxis for the sympathetic outflow to two distinct WAT pads, despite the differential sympathetic drives to WAT, differential degrees of lipid mobilization, and some shared circuits for WAT and BAT discussed above.

THE EXACT CIRCUITS AND BRAIN SITES RESPONSIBLE FOR ORCHESTRATING THE PHOTOPERIOD-INDUCED CHANGES IN BODY FAT ARE NOT KNOWN, BUT SOME LIKELY CANDIDATES HAVE BEEN IDENTIFIED

Given our knowledge of the SNS outflow from brain to WAT discussed above, and the failure to identify circulating factors responsible for the photoperiod-triggered increases in WAT lipid mobilization of Siberian hamsters also discussed above, we thought that perhaps MEL was interacting with the sympathetic innervation of WAT at some level(s) of the neuroaxis to increase the sympathetic drive (norepinephrine turnover [NETO]) to WAT (26). As noted above, MEL_{1a} receptors receive the nocturnal MEL durational signal that codes the photoperiod in Siberian hamsters as well as in other species (68, 69). Therefore, we tested whether MEL_{1a} receptor mRNA was colocalized with brain SNS outflow neurons to WAT labeled after PRV injections into the tissue, using emulsion autoradiographic in situ hybridization and immunohistochemistry, respectively (70). This seminal study was focused on forebrain only, but we realize that MEL binding/receptors have been localized in midbrain and hindbrain (e.g., median and dorsal raphe, raphe obscurus, and pontine reticular nuclei) in laboratory rats (71), areas that also contain neurons that are part of the sympathetic outflow to WAT (62, 67, 70, 72). Gene expression for the MEL_{1a} receptors is predominantly in the paraventricular and reuniens nuclei of the thalamus and in the SCN (70, 73, 74). The SCN is critical for the reception of season-encoded MEL signals, because pinealectomized Siberian hamsters bearing SCN lesions do not decrease their body or lipid mass or regress their gonads when given exogenously administered SD MEL signals (75–77). More importantly for our topic, there is extensive colocalization of MEL_{1a} receptor mRNA with PRV-labeled neurons in some brain sites involved in the sympathetic outflow from CNS to WAT, including the SCN (70). This suggests that stimulation of these receptors increases the sympathetic drive to WAT [as shown in SDs by increases in NETO (26)]. This, in turn, would initiate lipolysis and thereby drive the SD-induced increases in lipid mobilization by this species.

Unlike humans, in which increases in energy expenditure and/or decreases in energy intake trigger physiological responses to counter these attempts at reductions in adiposity (for review, see Refs. 78, 79), Siberian hamsters respond to SDs with a suite of coordinated sympathetic responses that work together to decrease body fat. Thus, in addition to the SD-induced increase in WAT NETO (26) discussed above, the potency/efficacy of norepinephrine (NE)-triggered lipolysis increases in a temporally and fat pad-specific manner (80). That is, during the rapid decrease in body fat occurring during the first 5–6 weeks of SD exposure, the potency (sensitivity/EC_{50}) and efficacy (maximal response asymptote) of NE-induced lipolysis was increased in isolated adipocytes from IWAT and EWAT compared with isolated adipocytes from their LD counterparts (80). These enhanced responses to NE were most prominent when lipid mobilization was greater (5 vs. 10 weeks of SD exposure) and were more marked in EWAT than in IWAT, paralleling the greater decrease in EWAT than in IWAT mass (80). Moreover, the SD stimulation of lipolysis was similar for NE and BRL 37344 (a specific β_3-adrenoceptor agonist), implying the primacy of this receptor subtype in this response (80) (see below for a brief discussion of β- and α-adrenoceptors in WAT). WAT β_3-adrenoceptor mRNA expression (and, thus, likely protein) also increases in SDs (81), suggesting that these increases in β_3-adrenoceptors could underlie the SD-induced increased NE sensitivity/efficacy. Collectively, therefore, there is a coordinated set of SD-triggered sympathetic responses that promote the seemingly effortless shift from the obese to the lean state in Siberian hamsters experiencing SDs.

To recapitulate, we have defined the likely sequence of events from environmental cue (change in photoperiod) to decreases in adipocyte size that result in the reversal of photoperiod-induced seasonal obesity in Siberian hamsters. Thus, with increasing durations of night (SDs), the peak duration of nocturnal MEL secretion by the pineal is lengthened, resulting in increases in MEL_{1a} receptor stimulation, some of which are located in the circuits constituting the sympathetic outflow neurons to WAT. This increase in the sympathetic drive to WAT stimulates primarily β_3-receptors that have increased efficacy/potency to NE, the principal sympathetic postganglionic neurotransmitter, as a result of SD exposure. This, in turn, initiates the lipolytic cascade that ultimately results in decreases in total body fat of SD-exposed Siberian hamsters.

We do not believe, however, that MEL interacting with the SNS in humans is an important factor controlling lipid mobilization. This does not diminish the importance of these findings, because it led us and others to realize the importance of the SNS innervation of fat for lipid mobilization. Moreover, as will be discussed below, the sympathetic innervation of WAT appears to be the underlying mechanism that initiates lipolysis under a number of conditions in both human and nonhuman animals.

DIFFERENTIAL SYMPATHETIC OUTFLOW TO PERIPHERAL TISSUES INCLUDING WAT IS THE RULE, NOT THE EXCEPTION

Walter Cannon (82) put forth the theory that at times of emergency, a general SNS discharge was triggered, preparing animals for "fight or flight." This "all-or-nothing" view of the sympathetic activation of peripheral tissues,

however, still appears to predominate, especially in the interpretation of SNS electrophysiological activity to peripheral tissues. For example, generalizations have been made and applied to WAT from recordings made of the sympathetic nerves innervating BAT, kidney, and other tissues. Unfortunately, the sympathetic activity to peripheral tissues is not analogous to the current flow to the electrical outlets in your home, where the current is virtually identical regardless of which outlet is measured. The physiological reality of sympathetic nerve activity is that it differs not only across tissues (e.g., heart vs. BAT) but within a type of tissue (WAT across its varied locations, as discussed below).

There have been several studies measuring the changes in the firing rate of sympathetic nerves innervating WAT, all by Niijima and associates (83–90), showing that a number of stimuli increase sympathetic drive to WAT, including odors, tastes, histamine, leptin, and other factors. This relative paucity of electrophysiological measures of sympathetic nerves to WAT contrasts with that for BAT (for reviews, see Refs. 91, 92), perhaps because the nerves to WAT are more difficult to identify and are smaller, making them more difficult to record from than the sympathetic nerves innervating BAT. In these studies of sympathetic nerve activity to WAT, this activity has not been measured in more than one WAT depot simultaneously (two EWAT pads have been measured simultaneously, but not, for example, EWAT and IWAT), and as we shall see, based on biochemical measures of sympathetic activity (NETO), differential sympathetic drive across WAT pads almost always occurs.

The deficit in our knowledge of sympathetic drives to WAT has been approached by measuring NETO as a proxy for direct electrophysiological measures. Most frequently, the α-methyl-para-tyrosine method has been used, in which α-methyl-para-tyrosine, a competitive inhibitor of tyrosine hydroxylase (TH), the rate-limiting enzyme in catecholamine biosynthesis, is exploited to allow the estimation of NETO by the rate of NE disappearance from the tissues of interest (93). Using this method, therefore, a measure of sympathetic drive can be compared across multiple tissue types and multiple adipose depots. When this is done, it is clear that there is differential NETO across WAT pads in response to many lipid-promoting stimuli. For example, as noted above, SD-exposed Siberian hamsters increase WAT NETO, with the more internally located WAT pads (RWAT and EWAT) showing the greatest increases compared with more externally located WAT pads (IWAT) (26). These SD-induced increases in NETO are accompanied by proportional decreases in WAT mass. Fasting does not significantly increase NETO differentially in laboratory rat RWAT or EWAT, although a trend for increased RWAT compared with EWAT NETO exists (94). By contrast, in 48 h fasted Siberian hamsters, NETO is significantly increased in IWAT but not in the other fat pads (M. Brito, N. Brito, C. K. Song, and T. J. Bartness, unpublished data). Similarly, although acute cold exposure does not produce significant differences in EWAT and RWAT NETO in laboratory rats, there is a trend toward increases in RWAT compared with EWAT NETO (95), and with acute cold exposure in Siberian hamsters, IWAT NETO is significantly greater than RWAT or EWAT NETO (no change) and dorsosubcutaneous white adipose tissue (DWAT) NETO (M. Brito, N. Brito, C. K. Song, and T. J. Bartness, unpublished data). Finally, another example of differential sympathetic drive across WAT pads occurs with the glucoprivic stimulus 2-deoxy-D-glucose: NETO is increased to several WAT pads (IWAT, RWAT, and DWAT) but not in EWAT (no change) in Siberian hamsters (9; M. Brito, N. Brito, C. K. Song, and T. J. Bartness, unpublished data). It should be noted that the relation between increases in NETO and decreases in WAT mass, as occurred in SD-exposed Siberian hamsters (26), does not always hold, as third ventricularly or peripherally administered leptin increases WAT NETO but is not always positively correlated with decreases in WAT mass (96). When such a disparity is seen, it may be because WAT pad mass is not a sensitive indicator of lipid mobilization when the degree of lipolysis is more subtle. Alternatively, receptor or post receptor events could be altered such that the sympathetic nerve activity is without effect. In summary, differential WAT NETO exists for several lipid-promoting stimuli, but our knowledge of the control of this and other trafficking of sympathetic drives to peripheral tissues is an important unsolved mystery that seems fundamental to solve for a deeper understanding of regulatory biology (for review, see Ref. 97).

THE NEUROCHEMICAL IDENTITIES OF NEURONS IN THE SYMPATHETIC OUTFLOW CIRCUITS FROM BRAIN TO WAT ARE LARGELY UNKNOWN, BUT A MAJORITY OF THESE NEURONS HAVE GENE EXPRESSION FOR THE MELANOCORTIN 4-RECEPTOR

In our previous studies, we found CNS neurons infected after PRV inoculation in WAT that also had colocalized immunoreactivity for arginine vasopressin (98), TH (98), oxytocin (98), acetylcholine transferase (72), and MEL_{1a} receptors (70), with the vast majority of cells not showing an identified neurochemical phenotype. Three possibilities for this finding exist. First, we simply did not test enough neurochemicals or receptor types for their presence in these neurons (see Ref. 98, however, for a long list of antibodies tested against neuropeptides and enzymes of synthesis that did not colocalize with PRV-infected neurons). Second, the viral infection inhibits the expression of neurochemicals. Third is a combination of both possibilities. High levels of colocalization can be found, however (see directly below), suggesting that it is possible for PRV-infected neurons also to be labeled for some highly expressed substances, specifically melanocortin 4-receptor (MC4-R) mRNA.

The melanocortins have been heavily implicated in the control of food intake and energy expenditure, largely through MC4-Rs (for review, see Refs. 99, 100). More specifically, MC4-Rs have been implicated in SNS-mediated

WAT lipolysis because central application of the MC3/4-R agonist, melanotan-II, in laboratory rats decreases food intake and body fat (101). These decreases in body fat are greater than can be accounted for by the decreases in food intake, as revealed by pair-feeding, suggesting increases in energy expenditure (101). We tested whether these increases in lipid mobilization were attributable to stimulation of MC4-Rs located on neurons that make up the sympathetic outflow circuits innervating WAT and found extensive colocalization of MC4-R mRNA with PRV-labeled SNS outflow neurons across the neural axis, with a high incidence and percentage (~60% or greater) for most brain areas showing PRV immunoreactivity (-ir) (67). These areas include the PVN, 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 nucleus, lateral reticular area, rostroventrolateral medulla, and anterior gigantocellular nucleus in the brainstem, to name a few of the more predominant sites of colocalization. This extensive high level of colocalization (~60%) suggests that MC4-Rs play a prominent role in the modulation of SNS outflow to WAT, either through stimulation by the endogenous melanocortin agonist α-melanocyte-stimulating hormone and/or through inhibition by the naturally occurring MC3/4-R inverse agonist, agouti-related protein (102). We have found similar high levels of colocalization of PRV with MC4-R mRNA sympathetic outflow to BAT (61; C. K. Song, E. Keen-Rhinehart, C. H. Vaughan, D. Richard, and T. J. Bartness, unpublished data), and while that work was in progress, a similar high level of colocalization was found after PRV injections into IBAT of MC4-R-green fluorescent protein transgenic mice (103). These studies demonstrate the usefulness of PRV in delineating the neurochemical phenotype of the sympathetic outflow neurons to WAT, BAT, or other tissues.

WAT DENERVATION STUDIES PROVIDE FUNCTIONAL EVIDENCE FOR THE ROLE OF THE SYMPATHETIC INNERVATION OF WAT IN LIPID MOBILIZATION

The notion that adrenal medullary EPI is the principal initiator of lipolysis was undermined because of the inability of adrenal demedullation (and thus no circulating EPI) to block lipid mobilization triggered by several stimuli that promote lipolysis (see above). Analogous experiments have been conducted to test whether destruction of the SNS innervation of WAT blocks lipid mobilization. Because most of the WAT pads studied by researchers are bilaterally located and unilaterally innervated (for review, see Ref. 9), the "unilateral denervation model" can be exploited. Specifically, in this model, one of a pair of WAT pads is denervated, with its contralateral mate serving as a within-animal neurally intact control that receives sham denervation. Therefore, all other characteristics of the animal are the same: genetics, age, energy balance, and all circulating factors except that one fat pad is denervated and the other is not. Such local surgical denervation offers more neuroanatomical specificity than does global sympathectomy using guanethidine (104) or 6-hydroxy-dopamine (6OHDA) (105); however, it is not neuroanatomically selective, as both sympathetic [there appears to be no or sparse parasympathetic nervous system (PSNS) innervation of WAT (72, 106), but see below for a discussion of this issue] and sensory nerves are severed. Indeed, surgical denervation significantly decreases TH-ir (a sympathetic nerve marker) and calcitonin gene-related peptide (CGRP)-ir (a sensory nerve maker), indicating reduced sympathetic and sensory innervations, respectively (107–109). The universal finding of these WAT denervation studies is that, regardless of the lipid-mobilizing stimulus, lipid mobilization is diminished or most often blocked by surgical denervation compared with neurally intact contralateral control pads. For example, fasting-induced decreases in WAT mass in laboratory rats, cats, rabbits, and dogs (110–114) are blocked, by surgical denervation, as is estradiol-induced decreases in WAT mass of ovariectomized rats (115) and the SD-induced decreases in WAT mass of Siberian hamsters (44, 116, 117). The lipid-mobilizing effects of physiological doses of leptin given peripherally, however, are not blocked by WAT sympathetic denervation in laboratory rats (53).

Complementary to the denervation studies are a few studies of sympathetic nerve stimulation (for review, see Ref. 11). In an ingenious yet simple study by Correll (118) conducted almost 45 years ago, the intact nerves innervating EWAT were electrically stimulated after the pads were removed with the nerves attached from laboratory rats and placed into a beaker of medium. Stimulation of the nerves markedly increased the FFA concentration in the incubation medium, suggesting lipolysis (118). This effect was blocked if the rats were sympathectomized 4–11 days before removal and the nerves to the pads were subsequently electrically stimulated in a similar manner (118). Moreover, adding dibenamine, a β-adrenergic blocker, to the incubation medium before the initiation of electrical stimulation blocked these increases in FFAs (119). Using the same preparation, others repeated and extended these findings, showing that another adrenergic receptor blocking agent [1-(2′,4′-dichlorophenyl)-1-hydroxyl-2-(*t*-butylamino)], a depletor of catecholamine stores (syrosingopine), and an inhibitor of NE release (BW 392C60) all blocked the electrical stimulation-induced increase in FFA concentration in the incubation medium (119). This electrical stimulation-triggered lipolysis is exaggerated by pargyline, a monoamine oxidase inhibitor (thus inhibiting NE degradation), and by theophylline, the phosphodiesterase inhibitor (119), the latter suggesting that endogenously released catecholamines are involved (119). In a relatively analogous in vivo preparation in humans, Dodt et al. (120–122) intraneurally stimulated the lateral femoral cutaneous nerve that innervates subcutaneous WAT and measured local lipolysis (changes in glycerol concentrations) via microdialysis of the interstitial subcutaneous WAT area receiving the stimulation. This stimulation increases interstitial glycerol con-

centrations in vivo in humans (120, 121). Thus, complementary electrical stimulation studies to the denervation studies discussed above also support a role of sympathetic innervation in initiating lipolysis in WAT.

ADRENOCEPTORS ARE INTIMATELY INVOLVED IN THE CONTROL OF LIPOLYSIS IN WHITE ADIPOCYTES

It is beyond the scope of this review to provide a detailed account of the current state of knowledge of the involvement of adrenoceptors in lipolytic responses or of endocrine/paracrine lipolytic factors; the reader is referred to several excellent reviews (123, 124). Although we will focus here on adrenergic effects on lipolysis, this is not to deny the importance of these endocrine/paracrine factors, such as natriuretic peptides [humans/primates only (125)], tumor necrosis factor-α, glucagon, and growth hormone (for reviews, see Refs. 123, 126). In addition to the adrenoceptor subtype α_2-adrenoceptor (see directly below), other nonadrenergic factors that inhibit lipolysis also are important, such as insulin, the most potent of these (127, 128), as well as prostaglandin E_2, adrenomedullin, adenosine (for review, see Ref. 123), and neuropeptide Y (129–132), the latter a neuropeptide that is often colocalized with NE in sympathetic nerves (133–135).

Pioneering work of Lafontan (136–139) and others (140–142) set the stage for our current knowledge of the role of adrenoceptors (adrenergic receptors) in lipolytic and other cell functions. In its simplest form, four subtypes of adrenoceptors have a role in catecholamine-stimulated lipolysis; these are the β_1-, β_2-, and β_3-adrenoceptor subtypes as well as the α_2-adrenoceptor (for reviews, see Refs. 124, 143–146). Activation of the three β-receptor subtypes stimulates lipolysis, but the participation of each subtype in lipolysis varies according to the fat pad, species, gender, age, and degree of obesity (147). By contrast, as noted above, activation of α_2-adrenoceptors inhibits lipolysis (for review, see Ref. 144). These four adrenoceptors coexist in adipocytes. Because activation of the β_1-, β_2-, and β_3-adrenoceptors stimulates lipolysis, whereas activation of the α_2-adrenoceptor inhibits lipolysis, it is not surprising that postreceptor events are different for each. Stimulation of the GTP binding protein G_s with β_1-, β_2-, and β_3-adrenoceptor agonism triggers increases in cAMP production by adenylyl cyclase that, in turn, stimulates protein kinase A, which, among other things, phosphorylates hormone-sensitive lipase (HSL) and perilipin (for review, see Refs. 123, 126, 148; see below for more on perilipin). By contrast, stimulation of the GTP binding protein G_i with α_2-adrenoceptor agonism triggers decreases in cAMP as a result of the inhibition of adenylyl cyclase and thus the lack of stimulation of protein kinase A and, therefore, the lack of phosphorylation of HSL and perilipin (for reviews, see Refs. 123, 126, 148).

Once this stimulation of β-adrenoceptors has begun, the process of hydrolyzing the stored triacylglycerol begins. Triacylglycerols in adipocytes are hydrolyzed in three steps, with HSL catalyzing triacylglycerol and diacylglycerol and monoglyceride lipase completing the process (for review, see Ref. 149). Perilipins interact with HSL and are positioned on the surface of lipid droplets within the adipocyte to block the translocation of HSL and thus inhibit catecholamine-induced lipolysis (for reviews, see Ref. 150, 151). With protein kinase A phosphorylation of perilipin and HSL, however, the phosphorylated HSL now has access to the lipid droplet and lipolysis proceeds (for reviews, see Ref. 126, 148). In addition, adipocyte lipid binding protein (or adipocyte fatty acid binding protein, A-FABP, 422 protein, aP2, and p15 protein) has been isolated, purified, and cloned in humans and rodents (152–155) and appears to assist in lipolysis. Adipocyte lipid binding protein accepts FFAs and activates HSL as well. Because adipocyte lipid binding protein removes FFAs from the cytosol, the well-known end product inhibition of lipolysis that occurs with accumulating FFAs seen in vitro (156) is negated in vivo, allowing lipolysis to proceed.

The preponderance of the data suggests that the balance between the lipolysis-promoting β-adrenoceptor activation and lipolysis-inhibiting α_2-adrenoceptor activation dictates the degree of lipolytic activity, all other factors being equal (for reviews, see Refs. 8, 145, 157). Thus, when β-adrenoceptor activation predominates, lipolysis is stimulated; conversely, when α_2-adrenoceptor activation predominates, lipolysis is inhibited (for reviews, see Refs. 143–145). The affinity of the naturally occurring agonists (EPI and NE) for these adrenoceptors is as follows, based on in vitro adipocyte membrane binding assays (145): for NE, $\alpha_2 > \beta_1 \geqslant \beta_2 > \beta_3$; for EPI, $\alpha_2 > \beta_2 > \beta_1 > \beta_3$. The presence of these receptors or their levels varies considerably across species, with humans, laboratory rats, and hamsters having ample α_2-adrenoceptors and laboratory mice having none (132).

It has been a physiological conundrum to understand how adipocytes can have receptors that stimulate lipolysis and those that inhibit lipolysis yet share the same agonists. It has been suggested that because of the higher affinity of the α_2-adrenoceptors compared with the β-adrenoceptors for low naturally occurring (basal) concentrations of NE or EPI, α_2-adrenoceptors are activated, thereby inhibiting lipolysis (148). This inhibition can be broken, however, with increases in concentrations of the agonists, especially NE released from the postganglionic sympathetic nerve terminals that impinge close to the adipocytes (148). Further evidence for the interplay between these receptors and a role of the α_2-adrenoceptor in obesity was shown in a clever genetic experiment in which β_3-adrenoceptor knockout mice also had human α_2-adrenoceptors expressed transgenically in their WAT (158). These animals became obese, but only with both high-fat diet feeding and the presence of the α_2-adrenoceptors (158).

The challenge for a pharmacological approach to obesity reversal through the stimulation of β_3-adrenoceptors has been to find a WAT-specific β_3-adrenoceptor agonist (despite the low numbers of this receptor subtype in

human WAT) that has a long-term, nonadapting stimulation of lipolysis without negative side effects, such as hypertension or stroke (for reviews, see Refs. 159, 160).

SNS INNERVATION OF WAT CAN CONTROL WHITE ADIPOCYTE PROLIFERATION

To this point, we have focused on the sympathetic control of lipid mobilization through the innervation of WAT. A lesser known and recognized function of the sympathetic innervation of WAT is the control of adipocyte proliferation. Despite hypercellularity being a hallmark of obesity (for review, see Ref. 161), relatively little research on fat cell proliferation has and is occurring relative to research on fat cell differentiation (for review, see Ref. 162), making the control of fat cell number (FCN) a clinically and physiologically significant unknown. Its importance in obesity is obvious, as increases in adiposity would be quite restrained, instead of being apparently limitless, if existing adipocytes merely filled to capacity with lipid. Although there are a host of circulating and paracrine factors that affect adipocyte proliferation (for review, see Ref. 162), there also is growing support for SNS innervation of WAT playing a highly significant part in this process. To our knowledge, the first unambiguous demonstration of the likely role of the sympathetics in fat proliferation was the inhibition of preadipocyte proliferation in vitro by physiological concentrations of NE (163). This effect is blocked by pretreatment of the preadipocytes with the β-adrenoceptor antagonist propranolol (163) and was possible because adipocyte precursor cells possess β-adrenoceptors (164). Therefore, if NE inhibits fat cell proliferation in vitro, then sympathetic denervation of WAT in vivo should stimulate fat cell proliferation. Indeed, surgical denervation significantly increases FCN in laboratory rat WAT (165).

We have since replicated this surgical denervation-induced increase in FCN several times in Siberian hamster WAT using the unilateral denervation model (63, 107, 109). A consistent finding of these studies is a denervation-induced increase in FCN but not FCS (63, 107). To more precisely determine whether it was the surgical denervation of WAT via the destruction of its sympathetic innervation or of its sensory innervation (see below), we surgically denervated WAT (positive control) or locally injected 6OHDA to kill only the sympathetic nerves and spare the sensory nerves or locally injected capsaicin to kill the sensory nerves and spare the sympathetics (107). Capsaicin is the pungent part of red chili peppers and also is a selective neurotoxin for unmyelinated sensory nerves (166, 167). We verified the destruction of the sympathetics by 6OHDA using immunohistochemistry for TH and the destruction of sensory innervation by capsaicin using immunohistochemistry for CGRP (107). Surgical denervation of WAT significantly decreased both TH-ir and CGRP-ir, as expected, and 6OHDA treatment significantly decreased TH-ir but not CGRP-ir, and both triggered an ~3-fold increase in FCN. By contrast, capsaicin significantly decreased CGRP-ir but not TH-ir and did not alter FCN (107). Collectively, these results point to the sympathetic innervation of WAT as inhibiting FCN.

Although FCN is increased by destruction of the sympathetic innervation of WAT, this does not necessarily translate into a denervation-induced increase in fat cell proliferation. We measure cellularity using a modification of the osmium tetroxide fixation method combined with Coulter Counter quantification of fat cells (168). With this method, adipocytes having diameters of <20 μm are literally screened out to eliminate lipid droplets and cell debris. Thus, denervation could cause an apparent increase in FCN through increased accumulation of lipid in small adipocytes by the elimination of basal lipolysis, thereby allowing them to be counted. To test for bona fide proliferation by sympathetic denervation, we recently (109) injected 6OHDA or capsaicin locally in WAT to selectively destroy the sympathetic innervation and spare the sensory innervation or to selectively destroy the sensory innervation and spare the sympathetic innervation, respectively. Surgical denervation was included as a positive control. To label proliferating adipocytes, we also injected bromodeoxyuridine (BrDU; a nonradioactive method of identifying dividing cells), and to determine whether the BrDU-labeled cells were adipocytes, we double labeled the tissue for AD3-ir, a white adipocyte-specific membrane protein (169, 170). Surgical denervation significantly decreased TH-ir and CGRP-ir, whereas 6OHDA treatment only significantly decreased TH-ir, but both treatments were associated with ~3- to 4-fold increases in BrDU+AD3-ir cells. By contrast, capsaicin treatment significantly decreased CGRP-ir but not TH-ir and was not associated with an increase in BrDU+AD3-ir (109). These results suggest that destruction of the sympathetic innervation of WAT stimulates preadipocyte proliferation (109). Collectively, it appears that increases in the sympathetic stimulation of β-adrenoceptors inhibits fat cell proliferation, whereas decreases in sympathetic stimulation of β-adrenoceptors promotes it.

Interestingly, the magnitude of the SNS denervation-induced increases in FCN (proliferation) appears to reflect the particular propensity of each WAT depot to grow by increasing FCN. Specifically, surgically denervated Siberian hamster IWAT achieves a greater percentage increase in FCN than similarly denervated RWAT (63), whereas EWAT surgical denervation does not affect FCN (107). This lack of denervation-induced increases in FCN by EWAT is completely consistent with the tendency of EWAT to increase its mass by increasing FCS, and the denervation-induced increases in FCN by RWAT and IWAT reflect the tendency of these pads to increase their masses primarily by increasing FCN, at least in the obesity associated with aging in Wistar rats (171). These effects of denervation also are consistent with the greater in vivo incorporation of radiolabeled thymidine into DNA in RWAT (a radioactive method of identifying dividing cells) than in EWAT from high-fat diet-fed laboratory rats (172). Therefore, it is easy to envision that a decrease in sympathetic drive to some of these WAT pads could promote

fat cell proliferation and thereby make significant contributions to increases in adiposity.

The underlying mechanisms triggering the sympathetic denervation-induced increase in fat cell proliferation are unknown beyond the decreases in the activation of adipocyte β-adrenoceptors (163). Mature adipocytes and their precursor cells have α_2-adrenoceptors, the function of which in mature fat cells is to inhibit lipolysis when stimulated (see above). The α_2-adrenoceptors, in turn, have been implicated in adipocyte proliferation (164, 173), because the surgical denervation of laboratory rat WAT is associated with increases in FCN that are preceded by increases in adipocyte α_2-adrenoceptor number (165). Stimulation of these receptors in denervated WAT could occur by any remaining sympathetic nerves or by adrenal medullary catecholamines (primarily EPI). Either of these alternatives seems possible because α_2-adrenoceptors show special affinity for low concentrations of EPI and to a lesser degree NE (see above). In neurally intact WAT, decreases in the sympathetic drive to WAT and consequent stimulation of fat cell proliferation seem to be associated with decreases in β-adrenoceptor number and increases in α_2-adrenoceptor number as the WAT pad mass expands (174). One way that α_2-adrenoceptor stimulation might trigger fat cell proliferation is through the local release of lysophosphatidic acid that is elicited by WAT α_2-adrenoceptor activation (173). Because lysophosphatidic acid added to preadipose cell lines triggers fat cell proliferation (173), one could imagine such an event occurring in vivo as well. This hypothesized role for α_2-adrenoceptors in fat cell proliferation is bolstered by the genetic addition of these receptors in β_3-adrenoceptor knockout mice fed a high-fat diet, in which their obesity is solely attributable to increases in FCN (158). Finally, decreases in sympathetic drive may stimulate the release of the recently discovered white adipocyte paracrine factor autotoxin, a type II ectonucleotide pyrophosphatase phosphodiesterase, that in turn could stimulate proliferation by its ability to release lysophosphatidic acid (175). Collectively, this scenario provides a possible mechanism by which fat cell proliferation is stimulated with decreases or abolition of its sympathetic drive.

THE PSNS INNERVATION APPEARS SPARSE AT BEST

Most but not all (e.g., peripheral blood vessels and sweat glands) tissues receive dual innervation by the autonomic nervous system. There is an initial report of WAT PSNS innervation (176), and a thorough discussion of those findings and the possibility of PSNS innervation of WAT were presented recently (72, 106, 177, 178). In that initial report (176), selective surgical denervation of the sympathetic innervation of WAT was thought to be achieved, thereby sparing the PSNS innervation. Although it is clear that separate neural provisions to WAT were indeed either severed or spared based on the researchers' intentions (54), irrefutable evidence of their respective associations with the SNS or PSNS remains to be demonstrated. Nevertheless, PRV was then injected into the presumed sympathetically denervated WAT pad to label the origins of the hypothesized remaining parasympathetic nerves. This resulted in extensive bilateral infection of the dorsal motor nucleus of the vagus, thereby laying the claim of extensive WAT PSNS innervation (176). Unfortunately, these remaining nerves were not labeled with any known neurochemical markers of the PSNS, nor were the parasympathetic ganglia that always accompany PSNS innervation of tissues/glands identified. Furthermore, the extensive bilateral dorsal motor nucleus of the vagus infection is questioned, as this area innervates most peripheral tissues unilaterally in rodents (179–182).

For these and other reasons (72, 106, 177, 178), we sought PSNS nerve markers in WAT (72). We used three types of animals, a standard mouse strain (C57BL mice), a genetically obese mouse strain (*ob/ob* mice), and a standard laboratory rat strain (Sprague-Dawley rats), examined three WAT pads (IWAT, RWAT, and EWAT), and tested for three previously proven neurochemical markers of PSNS innervation in other tissues (vesicular acetylcholine transporter, vasoactive intestinal peptide, and neuronal nitric oxide synthase). There was only one result: no labeling in any animal in any WAT pad for any established PSNS marker (72). Next, we selectively and locally sympathetically denervated WAT with 6OHDA, as shown by significantly decreased WAT NE content and TH-ir, with no effect on sensory innervation (CGRP-ir), thereby sparing the putative PSNS innervation (72). PRV was then injected into the 6OHDA- or vehicle-injected WAT several days later. PRV did not infect the sympathetic chain, spinal cord, or brain in any animal with 6OHDA-treated WAT, but vehicle-injected WAT inoculated with PRV had typical sympathetic chain, spinal cord, and brain viral infection patterns (72). These data showing a lack of significant or no WAT PSNS innervation are supported by older biochemical data showing no acetylcholinesterase, an enzyme important in the degradation of acetylcholine (183). Collectively, these findings suggest that PSNS innervation either does not exist or is relatively minor in its extent. If, however, convincing data supporting PSNS innervation emerge in the future, this would be interesting, as it would afford WAT the fine control of metabolism that other tissues/glands have with dual autonomic nervous system innervation.

WAT HAS SENSORY INNERVATION, THE FUNCTION OF WHICH REMAINS TO BE DEFINITIVELY IDENTIFIED

Because sensory innervation of tissues is the rule, not the exception, it should not be surprising that WAT has significant sensory innervation of WAT (for reviews, see Refs. 11, 64). The first study showing direct evidence of sensory innervation (i.e., tract tracing) was from Fishman and Dark (184); the anterograde tract tracer, True Blue, was applied to laboratory rat IWAT or DWAT, resulting in labeled neurons in the dorsal root ganglia (DRG), the

home of pseudounipolar ("bipolar") sensory neurons. These data are convincingly corroborated by histology performed at the level of the WAT pad, showing substance P-ir (185) and CGRP-ir in laboratory rat WAT (186). Substance P and CGRP are contained within, and released from, sensory neurons and thus are considered markers of sensory innervation (187). We extended these findings to Siberian hamsters, in which CGRP-ir occurred in IWAT and EWAT (107–109). Moreover, coculture of DRG cells with adipocytes from the 3T3-L1 cell line results in the stimulation of neurite outgrowth from DRG cells and is associated with increases in angiopoietin-1 and trkA, the former, a ligand for the endothelial cell-specific Tie2 receptor and the latter, a high-affinity receptor for nerve growth factor (188). Although electron microscopy did not indicate sensory nerve-adipocyte synapses, *en passant*-type sensory nerve-adipocyte interactions were observed (188). Collectively, these in vitro data further support the sensory innervation of WAT.

Although the pioneering study of Fishman and Dark (184) showed dorsal root ganglion pseudounipolar sensory neurons innervating WAT using traditional tract-tracing methodology, the reception sites in the brain for this sensory innervation are not labeled by this technique. We addressed this issue using a transneuronal viral tract tracer, the H129 strain of herpes simplex virus-1 [(HSV) generously donated by Dr. Richard Dix, Georgia State University], borrowing the approach that Rinaman and Schwartz (189) used to define the sensory inputs to the brain from the stomach. Unlike PRV, H129 initially seems to travel in a retrograde direction from the site of inoculation, in our case from IWAT, to the soma of the pseudounipolar sensory neurons in the DRG, where it replicates, but from that point on it only travels in an anterograde direction, such that the sensory afferent circuits to the brain become infected. It always has been puzzling how H129 would change direction after initial infection in the DRG, as other neural viruses always remain either unidirectional or bidirectional. This behavior may be more understandable with a different view of DRG pseudounipolar neurons. The famous neuroanatomist Cajal (190) describes the arm of the pseudounipolar neuron that reaches into the periphery as more dendrite-like than axonal; viewed in this manner, the H129 virus strain only travels anterogradely. Using standard immunohistochemistry, we found HSV-ir cells at all levels of the neuroaxis, including both the nodose ganglia (visceral afferents) and the dorsal horn of the spinal cord (spinal afferents), and in many of the classic autonomic output areas in the brainstem (rostroventrolateral medulla), midbrain (lateral periaqueductal gray, lateral parabrachial nucleus, and subcoeruleus), and forebrain (lateral hypothalamus, zona incerta, periventricular area, posterior hypothalamus, preoptic area, PVN and subparaventricular area, bed nucleus of the stria terminalis, and lateral septum), to name some of the more heavily infected sites. There also is a partial overlap of brain sites that receive sensory inputs from WAT with those contributing to the sympathetic outflow to WAT (C. K. Song and T. J. Bartness, unpublished data). Others reported that injection of cholera toxin B, a retrograde tracer, into retroperitoneal WAT (RWAT) labeled approximately four to seven cells in the nucleus gracilis of the brainstem but found no labeling in the dorsal horn of the spinal cord (they did not look at the DRG); thus, it appears that they may have labeled visceral sensory inputs but not spinal sensory nerves, although the design of the study was not strictly to define the origins of the sensory innervation of WAT.

The functional role of WAT sensory innervation is still largely unknown. Although leptin, the largely adipose-derived cytokine thought to convey adiposity information to the brain (191), is thought to exert many of its effects on energy balance by penetrating the brain and binding to receptors there (for reviews, see Refs. 192, 193), another periphery to brain conduit exists via sensory nerves. First, leptin receptors have been localized in nodose ganglion neurons in laboratory rats and humans (194), cell bodies of vagal afferents, and on the vagal trunk itself in laboratory rats (195). In addition, single vagal afferent activities from intestinal mechanoreceptors are electrophysiologically responsive to leptin in cats (196, 197). Moreover, injections of leptin into one laboratory rat EWAT pad triggers a dose-dependent increase in its sensory nerve afferent activity (83, 87) and elicits increases in sympathetic nerve activity to the contralateral EWAT pad, suggestive of a reflex arc (87). Given that peripheral and central leptin can increase the sympathetic drive to WAT, albeit nonuniformly (96), and that leptin can decrease adiposity independent of food intake (198, 199), the electrophysiological data from WAT sensory nerves after local leptin injection suggest receipt of the leptin signal by WAT sensory nerves, which, in turn, could trigger an increase in WAT sympathetic nerve activity that could potentially promote lipolysis (87). Finally, injection of leptin into perirenal WAT triggers increases in sympathetic nerve activity to the kidney, suggesting a broader enhancement of sympathetic drive than just to WAT with increasing WAT interstitial leptin concentrations (200).

To test the possible function of WAT sensory nerves, we turned to the lipectomy model, whereby the hypothetical body fat regulatory system is directly challenged by the removal of WAT pads (for review, see Ref. 201). When WAT is surgically removed, the remaining unexcised WAT pads increase their mass in what appears to be an attempt to compensate for the lipectomy-induced lipid loss. Compensatory responses have been seen in laboratory mice (202, 203) and rats (204–208), lambs (209), ground squirrels (210, 211), and Syrian (212, 213) and Siberian (19, 108, 214–218) hamsters, and suggestive evidence has been seen in humans (219, 220). The signal for this robust lipectomy-induced compensatory increase in WAT masses is unknown, but it could involve the disruption of WAT sensory innervation that accompanies lipectomy. Therefore, we compared the responses of nonmanipulated WAT pads after local and selective sensory denervation, accomplished by microinjecting capsaicin bilaterally into EWAT of Siberian hamsters (controls received vehicle injections) or by bilateral surgical EWAT lipectomy (controls received

sham lipectomy) (108). EWAT lipectomy triggered a significant increase in the masses of nonexcised WAT pads (i.e., RWAT and IWAT), as we have seen previously (19, 214–216). Remarkably, capsaicin produced the same magnitude increase in WAT mass by the same WAT pads even though there was no actual lipid deficit, just selective sensory denervation [the latter verified by the significantly decreased CGRP-ir but not TH-ir (108)]. Thus, although there was no lipid deficit, the response of the other WAT pads was to "compensate" for the perceived WAT loss and do so to the same degree as with real lipectomy (108). Therefore, two relatively straightforward conclusions can be gleaned from this work: *1*) the possible signal for the lipectomy-induced compensation of WAT removal is the ancillary removal of the sensory innervation of WAT that accompanies lipectomy; and *2*) a possible role of the sensory innervation of WAT is to inform the brain of the presence of WAT or of the size of the amount of stored lipid.

What the sensory nerves are monitoring in WAT is unknown at this time. We have seen increases in the electrophysiological responses of the sensory nerves from IWAT to von Frey hair stimulation (controlled tactile stimulation) of the pad, but not the surrounding tissue, in pilot work (G. J. Schwartz and T. J. Bartness, unpublished data), suggesting possible mechanoreceptors. Alternatively, and seemingly with more face validity, is the notion that the sensory nerves monitor local factors that reflect the state of the adipose tissue, such as the products of lipolysis (glycerol and FFAs) or factors that might correlate with the lipid content of the adipocytes, such as the cytokine leptin, among other possibilities. This remains to be tested. There are neural receptive elements in the gastrointestinal system that appear to respond to FFAs in sheep (221, 222) and laboratory rats (223), suggesting, at least, that such receptors exist.

PERSPECTIVES ON THE SYMPATHETIC AND SENSORY INNERVATION OF WAT

As described above, there is strong neuroanatomical, neurochemical, and functional evidence for WAT SNS innervation and much less so for the PSNS innervation of WAT. Strong neuroanatomical and neurochemical evidence also exists for WAT sensory innervation, and the function of these nerves may be to sense body fat levels. That stated, some questions involving the role of the SNS in lipid mobilization as well as its relevance to humans remain.

If the principal initiator of lipolysis is the sympathetic innervation of WAT, then several predictions can be made. First, denervation should block lipid mobilization, and indeed, plentiful and strong data are presented above showing that lipid mobilization is blocked with surgical denervation of WAT as well as with the more specific local chemical sympathectomy. Second, if activation of the WAT β-adrenoceptors by NE released from postganglionic sympathetic nerve terminals initiates the cascade of cellular events resulting in lipolysis, then elimination of the receptors (e.g., creating a WAT-specific β_3-adrenoceptor knockout mouse, given the primacy of this receptor subtype for lipolysis in rodents) also should block lipolysis. Indeed, initially, this seemed to be a simple story: the lipolytic response and stimulation of adenylyl cyclase activity in isolated white adipocytes of β_3-adrenoceptor knockout mice are absent or nearly so in response to EPI, as well as to β_1-, β_2-, and β_3-adrenoceptor subtype-specific agonists, indicating the primacy of the β_3-adrenoceptor in at least murine WAT lipolysis (224, 225). Therefore, if activation of β_3-adrenoceptors by SNS-released NE is the principal stimulator of lipolysis, then β_3-adrenoceptor knockout mice might be predicted to be obese as a result of the lack of the normal stimulation of lipolysis that occurs, for example, during the day in nocturnal rodents; however, they are not (225, 226). β_3-adrenoceptor knockout mice may simply have developmentally triggered compensatory responses, given that these knockouts were not time-dependent knockouts. Alternatively, as discussed in some detail below, there is no a priori reason to expect that the SNS innervation of WAT participates in both lipid mobilization and lipid accumulation through increases and decreases in sympathetic activity, respectively.

To address some of the compensatory system issues regarding developmental changes in other β-adrenoceptors in β_3-adrenoceptor knockout mice, as well as to identify other possible lipolytic mechanisms, the "β-less mouse" was genetically engineered (227). These mice exhibit a mild basal obesity (227, 228; cf. 229), but when fed a high-fat diet, they become severely obese, primarily as a result of a decrease in diet-induced thermogenesis (227). When fasted, the β-less mice have enhanced concentrations of glycerol and FFAs (enhanced lipolysis) compared with controls, perhaps as a result of increases in lipid mobilization by other lipolytic factors [e.g., glucagon (229)]. A more adipocyte-specific approach to the issue of β-adrenoceptors and lipolysis occurs in vitro with isolated white adipocytes from β-less mice (228). Basal lipolysis is no different between wild-type and β-less mice, and not surprising is the lack of lipolytic responses to β_1- and β_2-adrenoceptor agonists (228). There is, however, a 5-fold remaining lipolytic response to NE and the pan-β-adrenoceptor agonist isoproterenol as well as a 2-fold lipolytic response to CL316243, a specific β_3-adrenoceptor agonist (228). These and other data (228) suggest the possibility of developmental compensatory responses for the lack of β-adrenoceptors and/or an as yet unidentified β_4-adrenoceptor.

The data from the β-less mice suggest the possibility of nonadrenergic mechanisms involved in lipolysis, at least in highly derived genetically manipulated environments, although their contribution to day-to-day physiological lipolytic responses might be questioned. Nevertheless, other strongly held notions of adipocyte physiology that have attained dogmatic status also have been challenged to some degree by these genetic approaches. For example, the action of HSL in triacylglycerol hydrolysis was thought, in conjunction with monoglyceride lipase, to be the primary factor stimulated by catecholamines (see above and

Ref. 230). Generation of HSL knockout mice has undermined the sole importance of HSL in lipolysis, because food-deprived HSL knockout mice have a reduction in FFAs by ~40% from that seen in wild-type controls, but not a blockade of FFAs (231). Other lipases are involved, including adipose triglyceride lipase (232–234), which has high activity in the hydrolysis of triacylglycerol to diacylglycerol. Of the remaining hydrolysis of triglycerides in HSL knockout mice, estimates of the role of adipose triglyceride lipase have been made, suggesting that the vast majority of the remaining acylhydrolase activity is attributable to the activity of this enzyme, as shown by inhibiting adipose triglyceride lipase activity with adipose triglyceride lipase antibodies (234). It should be noted, however, that although a significant role of adipose triglyceride lipase occurs in the murine lipolytic cascade, in humans, it appears to have a lesser role (235, 236). Thus, there is a complicated intertwining of lipolytic proteins in lipolysis that requires further investigation, but even with its complexities and unknowns, catecholamine-induced lipolysis still appears to be the initiator of a significant portion of the lipolytic response and HSL is the only lipase affected by catecholamines.

Species-specific responses are prevalent in physiology, and the physiology of obesity is no exception. Several examples were given above, but as a conclusion to this review, we will examine how the sympathetic innervation of WAT plays a role in lipolysis for humans. An early suggestion that the sympathetic innervation of WAT is involved in lipid mobilization of human WAT was noted almost 100 years ago, when it was realized that paralyzed patients have increases in adiposity in their denervated extremities (237). Moreover, in a patient also afflicted with cancer cathexia, the mobilization of lipid occurs in the innervated leg, but not the denervated leg (237). Additional evidence for SNS involvement in lipid mobilization in humans has been reviewed by Dodt et al. (122). The most convincing evidence in humans for the involvement of the SNS in lipolysis are the increases of interstitial glycerol measured by in situ microdialysis in fully conscious humans after a behavioral treatment (e.g., mental or physical stress), with a lipolytic stimulus in the infusate (e.g., β-adrenoceptor agonist) or using intraneural electrical stimulation of the lateral femoral cutaneous nerve in combination with microdialysis of WAT to measure changes in glycerol and blood flow (120).

The SNS innervation of WAT could affect the distribution of body fat in humans, including the abdominal obesity more characteristic of men and the increased thigh and buttocks adiposity in women (31). In addition, certain pathological conditions associated with lipodystrophies also might have the SNS innervation of WAT as an underlying cause or contributor. For example, it was postulated (238) that the lipodystrophy accompanying the late stages of acquired immunodeficiency syndrome, the so-called human immunodeficiency virus (HIV)-associated adipose redistribution syndrome, may have increased sympathetic drive as its basis. Indeed, patients with HIV-1 infection and HIV-associated adipose redistribution syndrome have increased subcutaneous WAT NE concentration without increases in global sympathetic nerve activity (239).

There is some evidence of apparent sympathetic nerve dysfunction in human obesity. Intraneural stimulation of WAT in obese women does not trigger lipolysis in subcutaneous WAT, whereas the same stimulation produces a profound increase in lipolysis (interstitial glycerol concentrations measured by microdialysis) in their lean counterparts (121). These data suggest the potential for decreased SNS drive to WAT in obesity that could promote increases in adiposity. Such a suggestion should be taken cautiously, however, because these data were only obtained from one WAT pad, and in humans as with nonhuman animals (see above), there are fat pad-specific responses to lipolytic stimuli [abdominal subcutaneous WAT vs. femoral subcutaneous WAT (240)]. Contrary to the notion of decreases in sympathetic drive to WAT in obese humans, NE spillover (an index of total sympathetic activation) in the circulation of obese people is the same as that of lean individuals (241), a finding contradictory to the expected decrease in NE spillover in the obese versus lean humans. This global measure of sympathetic activity, however, obfuscates regional WAT differences in sympathetic drive as well as NE spillover from nonadipose tissues. Evidence for differential sympathetic drive across peripheral tissues in humans, albeit not WAT fat pads, comes from this same study; obese humans have increases in NE spillover from the kidney (241). It seems, however, that WAT samples from obese humans have increases in lipolytic activity (242) rather than decreases in lipolytic activity, as conventional wisdom might dictate. This likely is attributable in part or in whole to the increases in lipolysis by large-volume compared with small-volume fat cells (243–246). Increases in lipolysis by large fat cells seem contradictory to the maintenance of increased adiposity; however, the rates of reesterification of the released FFAs back into triacylglycerol are increased in obese compared with lean humans (247, 248). Thus, increases in sympathetic drive to WAT in obese individuals and the consequent increases in lipolysis appear to be countered by increases in reesterification rates compared with lean individuals.

Finally, the key analysis for the possible SNS involvement in obesity needs to be done in individuals as they are developing obesity, not in the more stable advanced obese state. In addition, although difficult to do in humans, multiple WAT depots, including visceral WAT, should be assayed so that we do not have to rely solely on measures in subcutaneous WAT depots, given that many if not most measures of WAT physiology and morphology differ among subcutaneous versus visceral WAT (and among depots within each type). Perhaps such studies could be done in conjunction with elective, low-risk surgeries. Once again, however, attempting to force the notion that changes in sympathetic drive to WAT must be the key mechanism for both lipid accumulation and lipid mobilization likely is inappropriate. The support for the role of the SNS innervation of WAT in lipid mobilization reviewed above is incontrovertible. The notion of de-

creases in WAT SNS activity promoting obesity, however, is controversial.

In conclusion, there is a heavy emphasis in the current research atmosphere on the importance of circulating factors, such as leptin, that may serve as afferent signals to the brain informing it of body fat levels. Similarly, from the efferent side, there continues to be some emphasis on adrenal medullary EPI as a means of mobilizing lipid from WAT. By contrast, this review suggests that in addition to, or in some cases in place of, these factors, the innervation of WAT is important. Strong and plentiful evidence was presented on the importance of the SNS innervation of WAT as the principal initiator of lipid mobilization. At this juncture, the sensory innervation of WAT is unquestionable, but its possible role in sensing some aspect of adiposity is intriguing.

This review is dedicated to the pioneering work of Drs. Renato Migliorini and Irving Faust, whose work greatly stimulated the thoughts of the senior author about the sympathetic innervation of WAT and fat cell proliferation, respectively. The authors thank Ruth Harris, Larry Swanson, James Granneman, Terry Powley, Gary Schwartz, Sheila Collins, Renato Migliorini, and Hans-Rudi Berthoud for discussion of the issues contained in this review. This research was supported, in part, by National Institutes of Health Grant R01 DK-35254 to T.J.B. and by the Center for Behavioral Neuroscience Viral Tract Tracing Core at Georgia State University, through the Science Technology Center Program of the National Science Foundation under agreement IBN-9876754.

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