Sympathetic innervation of white adipose tissue and its regulation of fat cell number

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Submitted 29 September 2003; accepted in final form 17 February 2004

Bowers, Robert R., William T. L. Festuccia, C. Kay Song, Haifei Shi, Renato H. Migliorini, and Timothy J. Bartness. Sympathetic innervation of white adipose tissue and its regulation of fat cell number. Am J Physiol Regul Integr Comp Physiol 286: R1167–R1175, 2004; 10.1152/ajpregu.00558.2003.—White adipose tissue (WAT) is innervated by the sympathetic nervous system (SNS), and the central origins of this innervation have been demonstrated for inguinal and epididymal WAT (iWAT and eWAT, respectively) using a viral transneuronal tract tracer, the pseudorabies virus (PRV). Although the more established role of this sympathetic innervation of WAT is a major stimulator of lipid mobilization, this innervation also inhibits WAT fat cell number (FCN); thus, local denervation of WAT leads to marked increases in WAT mass and FCN. The purpose of this study was to extend our understanding of the SNS regulation of FCN using neuroanatomical and functional analyses. Therefore, we injected PRV into retroperitoneal WAT (rWAT) to compare the SNS outflow to this pad from what already is known for iWAT and eWAT. In addition, we tested the ability of local unilateral denervation of rWAT or iWAT to promote increases in WAT mass and FCN vs. their contralateral neurally intact counterparts. Although the overall pattern of innervation was more similar than different for rWAT vs. iWAT or eWAT, its SNS outflow appeared to involve more neurons in the supracranial and solitary tract nuclei. Denervation produced significant increases in WAT mass and FCN for both iWAT and rWAT, but FCN was increased significantly more in iWAT than in rWAT. These data suggest differences in origins of the sympathetic outflow to WAT and functional differences in the WAT SNS innervation that could contribute to the differential propensity for fat cell proliferation across WAT depots in vivo.

body fat; cellularity; hyperplasia; pseudorabies virus; tract tracing; white fat

ENERGY BALANCE IS MAINTAINED through alterations in energy intake and energy expenditure. Lipid is the primary form of stored energy in mammals and is largely found in white adipose tissue (WAT) as triacylglycerols. Prolonged positive energy balance promotes obesity that in humans is associated with the general "metabolic syndrome" and type II diabetes (for review, see Ref. 44). Thus understanding how body fat is preferentially deposited and mobilized is necessary for the prevention and treatment of obesity, respectively.

One mechanism that may underlie the differential accumulation of lipid by WAT depots, as well as the differential mobilization of lipid, is through its innervation by the sympathetic nervous system (SNS) and the effects of its primary postganglionic neurotransmitter, norepinephrine (NE; for review, see Ref. 3). NE acting through the stimulation of WAT β-adrenoceptors is recognized as a major stimulator of lipolysis both in vitro and in vivo (for review, see Ref. 5). Thus regional differences in SNS innervation or drive may explain regional differences in adiposity via differential rates of lipolysis. The largely nonoverlapping populations of sympathetic postganglionic neurons innervating inguinal WAT (iWAT) and epididymal WAT (eWAT) in Siberian hamsters (46) provides neuroanatomical support for the existence of differential SNS innervation of WAT pads. In addition, there are some differences in the central circuits comprising the SNS outflow to these WAT depots as revealed by using a transneuronal viral retrograde tract tracer, the pseudorabies virus [PRV; e.g., medial preoptic area (MPA) has more infected neurons after PRV injections into eWAT than into iWAT (1)]. Moreover, there are differential SNS drives on WAT, as measured by NE turnover, that are correlated with lipid mobilization (46). Thus both neuroanatomical and functional evidence support the role of the SNS innervation of WAT and strongly implicate its role in the regional differences in WAT lipid mobilization, including in humans (e.g., see Ref. 13).

In addition to lipolysis, the SNS innervation of WAT also influences WAT cellularity. Specifically, increased sympathetic drive resulting in increased NE release appears to inhibit FCN, likely via an inhibition of fat cell proliferation. Thus addition of NE to white adipocyte precursor cells in vitro inhibits fat cell proliferation, an effect blocked by pretreatment with the general β-adrenoceptor antagonist propranolol (24). Furthermore, denervation of WAT triggers an impressive hypercellularity (~2-fold increase in FCN) in Siberian hamsters (47) and in laboratory rats (7), providing in vivo support for the necessarily associated with these health risks (38); rather it is the distribution of body fat that is critical (19). That is, visceral obesity is closely associated with development of the "metabolic syndrome" and type II diabetes (for review, see Ref. 44). Thus understanding how body fat is preferentially deposited and mobilized is necessary for the prevention and treatment of obesity, respectively.

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role of the SNS/NE in the control of FCN. Analogous to the differential mobilization of WAT via its SNS innervation may be a differential control of WAT fat cell proliferation by its SNS innervation, perhaps underlying the differences in the proliferative capacities of fat pads in vivo (12, 26), but this has not been tested.

Therefore, the purpose of the present study was to begin to test the role of the SNS as an underlying basis for the differential hypercellularity of WAT depots. In our previous studies, we focused on iWAT because of its great proliferative abilities in vivo, a characteristic not shared by the most frequently studied WAT depot, eWAT (for review, see Ref. 21). In the first experiment, we tested retroperitoneal WAT (rWAT), a pad also known for its proliferative abilities in vivo (12, 26), for the central origins of its SNS innervation by injecting it with PRV and then compared these results with those for iWAT and eWAT done by us previously in this species (1). In a second experiment, we tested for differences in the inhibition of FCN done by us previously in this species (1).

**METHODS**

**Animals and Housing**

Adult (>60 days) male Siberian hamsters (n = 31) were obtained from our laboratory breeding colony. The genealogy of this colony has been described recently (9) except that F2 generation wild-trapped hamsters were interbred with the colony in 1999 (through the generous gift of Stephan Steinelechner, School of Veterinary Medicine, Hannover, Germany). Hamsters were weaned at 21 days of age and housed with same-sex siblings. Before the initiation of the experiments (2 wk), animals were housed individually in polypropylene cages (27.8 × 7.5 × 13.0 cm) in colony rooms under a long day photoperiod (16:8-h light-dark cycle, lights on at 0200). Temperature was kept constant at 20°C, and relative humidity was maintained at 50 ± 5%. Food (Rodent Chow no. 3001; Purina, St. Louis, MO) and tap water were available ad libitum throughout the experiment. All experimental procedures were approved by the Georgia State University Institutional Animal Care and Use Committee in accordance with Public Health Service as well as United States Department of Agriculture guidelines.

**Experiment 1: Transneuronal Tract Tracing from rWAT to Brain**

**Surgical procedures and tissue harvesting.** Hamsters (n = 4) were anesthetized with pentobarbital sodium (30 mg/kg ip), an incision was made on the ventral side of the animal, and the left rWAT was exposed. In this study, we used PRV-152 (originally supplied as a generous gift of Dr. Lynn Enquist; Princeton University, Princeton, NJ, and subsequently propagated by Dr. Teryl Frey; Georgia State University, Atlanta, GA). PRV-152 is derived from PRV-Bartha, an attenuated strain of PRV-Becker, and PRV-152 carries the gene for enhanced green fluorescent protein (GFP) driven by the cytomegalovirus immediate early promoter (39). Briefly, a total of 300 nl PRV-152 (3 × 10⁶ plaque-forming units/ml) was injected in two areas of the left rWAT essentially as described previously (36). After PRV injection (6 days), the hamsters were deeply anesthetized with pentobarbital sodium (80 mg/kg ip) and perfused transcardially with 0.1 M sodium PBS containing 4% paraformaldehyde and 30% sucrose. Brains and spinal cords were removed and incubated in 4% paraformaldehyde overnight and then stored in the dark at 4°C in a solution containing 4% paraformaldehyde and 30% sucrose.

**Histological quantification of PRV-infected neurons.** Brains were sliced (30-μm sections) in the coronal plane using a freezing microtome, and slices were kept at 4°C in 0.1 M sodium PBS containing 0.1% sodium azide until mounted on slides. If forebrain infections were detected, brain stem and spinal cords were fully processed. Spinal cords were checked for conditions that would suggest nonspecific infections according to our standard procedures (e.g., Ref. 36). Briefly, the spinal cord of each infected animal was examined for lysis of the infected neurons. In addition, spread of the virus to the adjacent musculature was assessed by the presence of alpha motor neuron infections in the ventral horn of the spinal cord. Finally, noncontrolled spread of the virus was assessed by the presence of infections contralateral to the injection side in the spinal cord. Cases with lysis, alpha motor neuron labeling, or contralateral spinal cord infections are generally excluded; however, none of these situations occurred in the present study. Based on our previous studies, midbrain was not analyzed because the vast majority of infected neurons appear only in the periaqueductal gray area (1).

After being dried in the dark at room temperature, all slides were covered with anti-fading reagent (0.1 M n-propyl gallate in 0.05 M Tris-buffered saline) for preservation of GFP fluorescence. Fluorescent images from the microscope (BH-2; Olympus America, Melville, NY) were visualized using a video camera (PM20; Olympus America) and MagnaFire-SP software (version 1.0 × 5; Olympus America). Images were superimposed to previously scanned camera lucida drawings of Siberian hamster sections of the forebrain and brain stem (1), and the positions of PRV-infected neurons were marked on these images accordingly. The number of labeled neurons was counted in each nucleus throughout its complete rostral-caudal extent as it was done previously (1). We supply rostral-caudal measures relative to a rat stereotaxic atlas (33) to facilitate comparison to this more commonly studied species (i.e., forebrain sections: bregma −0.26 to −3.14 mm; brain stem sections: bregma −11.60 to −14.60 mm).

**Experiment 2: WAT Mass and Cellularity After Surgical Denervation**

In this experiment, either iWAT or rWAT was surgically denervated, and NE content and cellularity were determined 5 wk later.

**Surgical procedures.** Because there are no differences in the mass of the left and right rWAT, as assessed from age- and body mass-matched male hamsters [left rWAT = 0.097 ± 0.014 (SE) g, right rWAT = 0.110 ± 0.014 g, unpublished data], the left rWAT was denervated, and the right pad served as an unoperated control. Thus hamsters [rWAT (n = 15) and iWAT (n = 12)] were anesthetized with pentobarbital sodium (50 mg/kg), and an incision was made on the ventral side of the hamster above the left kidney. The kidney was gently lifted, and the three nerve bundles were cut immediately before they reached the fat pad, allowing the rWAT pad to be undisturbed during the surgeries. Unilateral inguinal fat pad denervation was performed as previously described (47). For both surgeries, the contralateral pad was left intact and used as a within-animal control as we have done previously (10, 11, 47).

**WAT NE content.** After the denervation surgeries (5 wk), the SNS denervation of rWAT and iWAT was verified by measuring NE content using reverse-phase HPLC with electrochemical detection following our modification [of the method of Melford (31); see Ref. 46]. Briefly, a sample of rWAT (~25 mg) or of iWAT (~100 mg) was thawed and homogenized in a solution containing 10 ng dihydroxybenzylamine (internal standard) in 0.2 M perchloric acid with 1 mg/ml ascorbic acid (PCA-AA). After centrifugation at 7,500 g for 10 min, catecholamines were extracted from the homogenate with alumina and eluted into PCA-AA. Catecholamines were assayed using an ESA (Bedford, MA) HPLC system with electrochemical detection (Cuolochem II). The mobile phase was Cat-A-Phase II, and the column was a C₁₈ reverse-phase column. Results are expressed as nanogram NE per gram WAT.

**Determination of cell number and size distribution.** A sample of rWAT (~50 mg) or iWAT (~100 mg) was fixed in osmium tetroxide according to the method of Hirsch and Gallain (23). Cell number and size distribution were determined by Coulter Counter analysis (Beckman Coulter, Fullerton, CA), as previously described (47).

AJP-Regul Integr Comp Physiol • VOL 286 • JUNE 2004 • www.ajpregu.org

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Statistics

For experiment 1, the number of GFP-labeled neurons after PRV injection in rWAT (present study), iWAT and eWAT (previous study; see Ref. 1) was analyzed by Mann-Whitney Rank Sum Tests that compare nonparametric ranks of values. For experiment 2, means and SE of the left and right measurements of NE content, FCN, FCS, and fat pad mass obtained within the same animal were compared by Student’s paired t-tests. For all tests, differences among means were considered significant at P < 0.05. Exact probabilities and test values have been omitted for simplification and clarity of the presentation of the results.

RESULTS

Experiment 1

Transneuronal tract tracing from rWAT to brain. All four hamsters injected with PRV in the left rWAT became infected, but no indication of nonspecific infection, as described above, was seen in the spinal cords. The pattern of PRV infection in all hamsters was highly similar, and infected neurons could be visualized throughout the brain stem, midbrain, hypothalamus, and forebrain.

Distribution and quantification of PRV-infected neurons. Table 1 shows the average number of infected neurons in the forebrain and brain stem 6 days after PRV injection in rWAT. PRV-infected neurons were found at all levels of the brain stem and forebrain. Infected neurons were found bilaterally, with a greater density of labeling ipsilateral to the side of injection at virtually all sites. Representative photomicrographs of hypothalamic and brain stem PRV infections are shown in Fig. 1, a–c and d–g, respectively.

Brain stem. At caudal levels of the brain stem (bregma = −14.30 to −13.45 mm), most PRV-infected nuclei were located in the ventral part of the brain stem, including the lateral reticular nucleus and dorsal and ventral aspects of medullary reticular nuclei (Fig. 2 and Table 1). Only a few PRV-infected neurons were found in the raphe pallidus nucleus at this level (Table 1). The nucleus of the solitary tract was the most infected dorsally located area (Figs. 1d and 2 and Table 1).

At the level of the primary aspect of rostroventrolateral reticular nucleus (RVL) where the CI epinephrine cells reside (bregma = −13.30 to −12.72 mm), intense PRV labeling was seen in the RVL (Fig. 1e) and more centrally, including all aspects of the gigantocellular nucleus and the raphe obscurus nucleus (Fig. 1, f and g), as well as intermediate reticular (IRt) and parvocellular reticular nuclei (PCRt; Fig. 2 and Table 1).

At rostral levels of the brain stem (bregma = −12.30 to −11.60 mm), PRV-infected neurons mostly were found in the RVL and lateral paragigantocellular nucleus (Fig. 2 and Table 1). The most infected dorsal nucleus was the medial vestibular nucleus. Some PRV-infected cells also were seen in the IRt and PCRt (Fig. 2 and Table 1).

In terms of comparing the overall patterning of PRV-infected neurons in the brain stem of rWAT-injected hamsters in the present study vs. our earlier studies of iWAT and eWAT (1), we conducted a nonparametric analysis of structures that had PRV-infected neurons in both studies. No significant differences in the distribution of infected neurons among these WAT pads resulted, suggesting that the sympathtic outflow from brain to these pads is more similar than different at this level of the neuroaxis.

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<th>Table 1. Number of transneuronally labeled neurons after PRV injection in rWAT</th>
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Values are means ± SE. ARC, arcuate hypothalamic nucleus; BST, bed nucleus of the stria terminalis; CI, caudal interstitial nucleus; Cu, cuneate nucleus; CVL, caudoventrolateral reticular nucleus; DA, dorsal hypothalamic area; DM, dorsomedial hypothalamic nucleus; Gi, gigantocellular reticular nucleus, main aspect; GiA, gigantocellular reticular nucleus, alpha part; GiV, gigantocellular reticular nucleus, ventral part; Irt, intermediate reticular nucleus; LH, lateral hypothalamic area; LPGi, lateral paragigantocellular nucleus; LPO, lateral preoptic area; LRt, lateral reticular nucleus; MdV, medullary reticular nucleus, ventral part; MPA, medial preoptic area; MPO, medial preoptic nucleus; MVe, medial vestibular nucleus; PCRt, parvocellular reticular nucleus; PrH, prepositus hypoglossal nucleus; PVN, paraventricular hypothalamic nucleus; ROh, raphe obscurus nucleus; RPa, raphe pallidus nucleus; RVL, rostroventrolateral reticular nucleus; SCN, suprachiasmatic nucleus; Sol, nucleus of the solitary tract; ZI, zona incerta.

Forebrain. At the caudal hypothalamus at the level of the ventromedial hypothalamic nucleus (VMH; bregma = −3.14 to −2.12 mm; Fig. 2 and Table 1), PRV neurons were seen in the lateral hypothalamus (LH), zona incerta, arcuate hypothalamic nucleus, as well as dorsal hypothalamic area and dorsomedial hypothalamic nucleus (Fig. 2 and Table 1). No infected neurons were seen in the VMH. In terms of more dorsally located areas of the diencephalon, only a few PRV-infected cells were seen in the posterior part of the paraventricular hypothalamic nucleus (PV; Table 1).

More rostrally (bregma = −1.80 mm; Fig. 2 and Table 1), the majority of infected neurons was in the medial parvicellular and magnocellular portions of the hypothalamic paraventricular nucleus (PVN). At the level of the suprachiasmatic nucleus (SCN; bregma = −1.30 to −0.92 mm), the infected neurons were seen in the SCN (Fig. 1, a and c), anterior parvicellular PVN (Fig. 1, a and b), MPA, and anterior hypothalamic area and LH area (Fig. 2 and Table 1).
Continuing rostrally in the forebrain (bregma = −0.82 to −0.26 mm), both MPA and the medial preoptic nucleus were heavily labeled with PRV. A few labeled cells were seen in the lateral preoptic area (Fig. 2 and Table 1). In addition, some PRV cells were seen in the medial and ventral divisions of the bed nucleus of the stria terminalis (Fig. 2 and Table 1).

In terms of comparisons of the overall patterning of labeling for PRV-infected neurons in the forebrain of rWAT-injected hamsters in the present study vs. our earlier studies for iWAT and eWAT (1), the nonparametric analysis of structures that had PRV-infected neurons in both studies suggested that the sympathetic outflow from brain to rWAT vs. iWAT was significantly different.
The basis for this significant difference appears to be greater involvement of the SCN and the nucleus tractus solitarius for rWAT than iWAT (Table 1 and Ref. 1).

It should be noted that a better method to determine the viscerotopic distribution of SNS outflow neurons innervating different WAT pads requires multiple isogenic viruses that contain insertions of genes encoding distinct reporters (e.g., Ref. 15), and studies are ongoing to develop this procedure for this purpose. The comparisons made in the present study, however, afford the only method for such comparisons at this time and are considered suggestive.

Experiment 2

WAT mass and cellularity increased after surgical denervation. At the end of this experiment, NE content was determined to verify the SNS denervation. NE content was significantly decreased in rWAT and iWAT after denervation (P values < 0.05; Fig. 3A), and both denervation surgeries were equally effective. The mean ± SE percent difference in NE content for innervated vs. denervated tissue was −64 ± 4.38% for iWAT and −62.66 ± 2.81% for rWAT.

Postsurgery (5 wk), fat pad mass significantly increased in denervated pads compared with their sham contralateral counterparts for both rWAT and iWAT (P values < 0.05; Fig. 3B). Although the percent increase in mass for denervated vs. sham-operated control pads appears larger in iWAT than rWAT, this difference did not reach statistical significance (P = 0.06).

In rWAT, surgical denervation caused a significant increase in FCN for cells of the 25- to 50-µm and 75- to 100-µm ranges (P values < 0.05; Fig. 4A); and in iWAT, surgical denervation caused a significant increase in FCN for cells of the 25- to 50-µm, 50- to 75-µm, and 75- to 100-µm ranges (P values < 0.05; Fig. 4B). The mean cell diameter was not different

Fig. 2. Schematic diagrams showing the location of PRV neurons in the sections of the brain stem and forebrain from caudal to rostral levels in Siberian hamsters 6 days after PRV injection in left retroperitoneal (r) white adipose tissue (WAT). Bregma measurements copied from the rat atlas (40) are at the top right of each section. Each symbol represents 1–5 PRV-infected neurons. 12, Hypoglossal nucleus; 4V, 4th ventricle; ac, anterior commissure; AHA, anterior hypothalamic area, anterior part; AHc, anterior hypothalamic area, central part; Arc, arcuate hypothalamic nucleus; BSTMA, bed nucleus of the stria terminalis, medial division, anterior part; BSTV, bed nucleus of the stria terminalis, ventral division; C3, C3 adrenaline cells; CC, central canal; cc, corpus callosum; Cu, cuneate nucleus; CVL, caudoventrolateral reticular nucleus; DA, dorsal hypothalamic area; DM, dorsomedial hypothalamic nucleus; f, fornix; Gi, gigantocellular reticular nucleus; GiA, gigantocellular reticular nucleus, alpha part; GiV, gigantocellular reticular nucleus, ventral part; Irt, intermediate reticular nucleus; LH, lateral hypothalamic area; LGi, lateral paragigantocellular nucleus; LPO, lateral preoptic area; LRT, lateral reticular nucleus; LV, lateral ventricle; MDD, medullary reticular nucleus, dorsal part; MIV, medullary reticular nucleus, ventral part; MPA, medial preoptic area; MOE, medial preoptic nucleus; MV, medial vestibular nucleus; ox, optic chiasm; PCr, parvo-cellular reticular nucleus; PMn, paramedian reticular nucleus; PrH, prepositus hypoglossal nucleus; PV, paraventricular thalamic nucleus; PVPN, paraventricular hypothalamic nucleus; RPB, paraventricular thalamic nucleus, posterior part; Re, reuniens thalamic nucleus; RMG, raphe magnus nucleus; ROb, raphe obscurus nucleus; RPa, raphe pallidus nucleus; RVL, rostroventrolateral reticular nucleus; sol, nucleus of the solitary tract; sp5, spinal trigeminal tract; VMH, ventromedial hypothalamic nucleus; ZI, zona incerta.
between denervated rWAT or iWAT and their sham-operated counterparts (Fig. 4), however. Surgical denervation produced a significantly greater increase in total FCN in iWAT compared with rWAT (%increase in FCN caused by denervation: iWAT 120.83% ± 28%, rWAT 55.1% ± 15.2%).

**DISCUSSION**

The purpose of the present study was to serve as a beginning to test if the SNS innervation of WAT underlies the tendency for some WAT pads to increase FCN in vivo more than others under conditions that promote body fat accretion. This hypothesis is predicated on the differential innervation of WAT depots by the SNS. We previously demonstrated largely non-overlapping populations of postganglionic sympathetic neurons innervating iWAT and eWAT in Siberian hamsters (46), a finding that is not surprising given the disparate rostral-caudal locations of these fat pads. In the first experiment of the present study, we tested for differences in the central circuitry involved in the SNS outflow from brain to WAT pads using the viral transneuronal tract tracer PRV. We found that, although the overall pattern of innervation of rWAT (present experiment) was more similar than different compared with that of eWAT or iWAT (1), the SNS outflow to rWAT appeared to involve more neurons from the suprachiasmatic and solitary nuclei than for these other pads. The differential control of FCN by the sympathetic innervation of WAT was tested by removing the SNS inhibition of WAT FCN (7, 24, 46) through local surgical denervation. Although we found significant increases in WAT mass and FCN for both rWAT and iWAT, the denervation-induced increase in FCN was significantly greater for iWAT than for rWAT. Collectively, these findings, together with those from our previous studies on the SNS outflow from brain to eWAT and iWAT (1, 36, 41), and the findings by others (7) and by us on the inhibition of FCN by the SNS/NE (46), further strengthen the notion that the SNS innervation of WAT contributes to the control of FCN.

Although the surgical denervations in the present study, and surgical or chemical denervations in our previous work (10, 11, 47) and that of others (7) were not complete, they still produce impressive increases in fat pad mass and FCN (an approximate doubling); therefore, it is easily imagined that a complete SNS denervation, if possible, would produce even greater responses unless the physiological capacity to do so has already been met.

In the present study, FCS was not affected in rWAT or iWAT 5 wk after surgical denervation, corroborating the results of our previous study demonstrating a similar lack of change in FCS after surgical denervation of iWAT (47). In contrast, the present data, together with those by others (7) and by us (46), demonstrated that surgical denervation causes
increases in FCN. None of these studies, however, including the present one, discriminated between increased FCN resulting from bona fide preadipocyte proliferation vs. differentiation of preadipocytes into mature fat cells. It appears that mitotic clonal expansion precedes preadipocyte differentiation, so a combination of both processes may be involved (43). To determine which or both of these processes underlies the denervation-induced increased FCN, a different approach is required, such as in vivo labeling of proliferating cells with \[^{3}H\]thymidine (e.g., see Ref. 25) or bromodeoxyuridine (e.g., Ref. 22). Indeed, we have preliminary data showing that denervation induces an increase in bromodeoxyuridine-labeled cells that also are labeled with an antibody specific to a white adipocyte-specific membrane protein, thus demonstrating that these cells are the result of bona fide fat cell proliferation (M. T. Foster and T. J. Bartness, unpublished observations).

What is the mechanism underlying the differential denervation-induced increase in FCN in the present experiment and the general increase in FCN after surgical denervation seen previously (7, 46)? Although the SNS outflow from brain to WAT appears more similar than different among rWAT (present experiment) and iWAT and eWAT (1) in Siberian hamsters, there are some apparent differences in the pattern of the central origins of this innervation. We should note, however, some caveats regarding comparisons between the present study and our past work (1). Among the differences between these studies is the use of a larger virus titer in the present study than in the previous study (1) and that different investigators performed the quantification of infected cells, although the same microscopy procedure was used. Despite these caveats, rWAT appears to have more marked innervation (%infected cells) from the SCN of the hypothalamus and from the nucleus of the solitary tract in the brain stem than does eWAT or iWAT (1). Therefore, the potential exists for separate control by the central nervous system (CNS) of the SNS drive on WAT at these distal levels of the neuroaxis and thus a possible neuroanatomical basis for the differential inhibition of WAT cell proliferation.

In addition to the SNS innervation of WAT, this tissue also receives sensory innervation (18, 20) and perhaps parasympathetic nervous system innervation (2, 28). These nerves likely would be severed with the surgical denervation of WAT. Specifically, in terms of the sensory innervation of WAT, the evidence for such innervation is convincing deriving from tract tracing experiments showing retrograde labeling of neurons in the dorsal root ganglia after retrograde tracers are placed in WAT(18) and from immunocytochemical analyses of peptides associated with sensory nerves (i.e., calcitonin gene-related peptide and substance P; see Ref. 20). The role of this innervation for WAT growth and metabolism presently is not known, but in laboratory rats that have global peripheral sensory denervation induced by systemic injection of the sensory neurotoxin capsaicin, FCN is decreased rather than increased (8), suggesting that elimination of the sensory innervation of WAT does not promote increases in FCN as seen in the present study with surgical WAT denervation. Global sensory denervation is nonspecific, eliminating sensory innervation of all organs, not just WAT and thereby might produce numerous physiological changes that could obfuscate any role of the sensory innervation of WAT in FCN. Preliminary data where capsaicin is locally injected in WAT to produce restricted and selective sensory denervation suggest that WAT pad mass did not change after this treatment nor did FCN (37). Thus the elimination of sensory nerves to WAT as an inevitable consequence of surgical denervation does not seem likely to account for the changes in WAT mass and FCN seen in the present study.

A final possible mechanism underlying the effect seen here is the proliferative ability of tumor necrosis factor-\(\alpha\) (TNF-\(\alpha\)) that has been shown to stimulate fat cell proliferation in vitro (27). If involved, we believe the induction of TNF-\(\alpha\) would be through a different role than its function in inflammatory responses, as we had minimal contact with the fat pads during the denervation surgery and there were no overt signs of inflammation 5 wk later. Alternatively, TNF-\(\alpha\) release may be controlled by the SNS innervation of WAT. Stimulation of \(\beta\)-adrenoceptors with the \(\beta\)-adrenoceptor agonist isoproterenol inhibits TNF-\(\alpha\) production, whereas blockade of these receptors stimulated its production (14); thus, denervation may dishibit any SNS inhibition of TNF-\(\alpha\) production by WAT and therefore lead to an increase in FCN.

A possible cellular means by which the SNS could modulate FCN in WAT is via the stimulation of specific WAT adipocyte adrenoceptor subtypes. That is, WAT cell membranes possess both \(\alpha\)- and \(\beta\)-adrenoceptors, with the specific \(\alpha\)- and \(\beta\)-subtype and receptor number varying across species and among WAT pads within a species (for review, see Ref. 30). With regard to the role of the SNS in FCN/proliferation, NE inhibits rat WAT precursor stromal vascular cell proliferation in vitro, and this inhibition is through \(\beta\)-adrenoceptors because pretreatment with the non-selective \(\beta\)-adrenoceptor blocker propranolol removes the NE-induced inhibition of precursor stromal vascular cell proliferation in vitro (24). There also is considerable evidence for interaction between \(\beta\)-adrenoceptors and the MAP kinase pathway in adipocytes (e.g., Ref. 40), a pathway known to be involved in cell proliferation in other tissues (for review, see Reg. 6). By contrast, there is evidence for \(\alpha\)-adrenoceptor receptors influencing adipocyte proliferation, especially \(\alpha_2\)-adrenoceptors (4, 45). Both Syrian and Siberian hamsters and humans possess significant amounts of the functional antilipolytic \(\alpha_2\)-receptors, whereas laboratory rats and mice apparently do not (for review, see Ref. 29). Consistent with the possible involvement of this receptor subtype in controlling FCN, chemical denervation increases white adipocyte \(\alpha_2\)-adrenoceptor number, which precedes a striking increase in FCN (7), and this increase is not the result of fat cell differentiation (35). The one way by which these adipocyte \(\alpha_2\)-adrenoceptors could be stimulated in sympathetically denervated WAT is through the compensatory increase in the release of adrenal medullary catecholamines that follows sympathectomy (42). Thus it seems possible that stimulation of these receptors might trigger hyperplasia. The mechanism by which \(\alpha_2\)-adrenoceptor stimulation increases fat cell proliferation is not well understood, but locally released lysophosphatidic acid (45), a glycerophospholipid (32), may be involved. Application of \(\alpha_2\)-adrenoceptor agonists to white adipocytes triggers a rapid and prolonged release of lysophosphatidic acid (45). Moreover, application of lysophosphatidic acid to preadipose cell lines triggers fat cell proliferation (45). It may be that the newly discovered white adipocyte paracrine factor autotoxin, a type II ecto-nucleotide pyrophosphatase phosphodiesterase, stimulates proliferation via lysophosphatidic acid (17).
Collectively, the results of the present study suggest the components of the CNS outflow neurons of the forebrain and brain stem to rWAT are more similar than different compared with iWAT and eWAT, although the caveats listed above temper that conclusion. Functionally, in terms of the SNS inhibition of proliferation/FCN, there is a greater inhibition in rWAT than in iWAT, at least in this species.

ACKNOWLEDGMENTS
We thank Drs. Lynn Enquist for supplying the PRV-152, Teryl Frey for growing the virus, and Michael Wesley for assistance with the histology. We also thank David Marshall and Quijung W. Po for expert animal care.

GRANTS
This work was supported by National Institute of Diabetes and Digestive and Kidney Diseases Research Grant R0-1 DK-35254 and by the National Science Foundation Center for Behavioral Neuroscience Systems Core.

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