Sympathetic denervation does not prevent a reduction in fat pad size of rats or mice treated with peripherally administered leptin

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IT IS WELL ESTABLISHED THAT administration of leptin to experi-
or mice treated with peripherally administered leptin

Sympathetic denervation does not prevent a reduction in fat pad size of rats or mice treated with peripherally administered leptin. Leptin increases sympathetic nervous system (SNS) activity in brown adipose tissue and renal nerves. Experiments described here tested whether SNS innervation is required for peripheral, physiological concentrations of leptin to reduce body fat. In experiment 1, one epididymal (EPI) fat pad was sympathectomized by local injection of 6-hydroxydopamine (6OHDA) in C57BL/6 mice that were then infused for 13 days with PBS or 10 μg leptin/day from an intraperito-
nal miniosmotic pump. Surprisingly, EPI denervation increased total body fat of PBS-infused mice but leptin decreased the size of both injected and noninjected EPI pads in 6OHDA mice. Experiment 2 was identical except for the use of male Sprague-Dawley rats that were infused with 50 μg leptin/day. Leptin had little effect on EPI weight or norepinephrine (NE) content, but denervation of one EPI pad decreased the effect of leptin on intact EPI, inguinal and retroperito-
nal (RP) fat and increased the size of the mesenteric fat pad. Experiment 3 included groups in which either one EPI or one RP pad was denervated. RP denervation reduced RP NE content but did not prevent a leptin-induced reduction in fat pad mass. Therefore, the SNS is not required for low doses of leptin to reduce body fat. EPI denervation significantly increased adipocyte number in contralateral EPI and RP fat pads and this was prevented by leptin. These changes in intact pads of rats with one denervated fat pad imply communic-
tion between fat depots and suggest that both leptin and the SNS regulate the size of individual depots.

peripheral leptin infusion; norepinephrine; body weight

have shown that very high concentrations of leptin promote lipolysis from adipocytes in wild-type mice (15) and rats (16), whereas physiological concentrations of leptin (10 ng/ml) have no effect on the lipolytic rate in isolated adipocytes (16). The lipolysis that is induced by leptin is unusual because a simul-
taneous stimulation of fat cell fatty acid oxidation results in selective release of glycerol (41). An alternative way in which leptin could reduce body fat is by reducing the number of fat cells present. The small amount of information that is available on the effects of leptin on preadipocyte growth is inconsistent, suggesting either no effect (2) or a stimulatory effect (29) of leptin on preadipocyte proliferation and differentiation, whereas others have reported that centrally administered leptin increases adipocyte apoptosis in vivo (34).

Because fat cells express both long- and short-form leptin receptors (13, 17), the hormone may act directly on the tissue to modify metabolism or leptin could potentially modify activity of another metabolically active factor, such as insulin, either by changing the release of the factor or by interfering with its signaling at the fat cell. This is easy to imagine as leptin and insulin activate common proteins in their postrecep-
tor signaling pathways (28). Alternatively, leptin from the periphery may cross the blood-brain barrier and activate central regulators of sympathetic nervous system (SNS) outflow to white adipose tissue (WAT). Although leptin administration has been shown to increase sympathetic activity in intrascapu-
lar brown adipose tissue (IBAT) (7) and in renal lumbar nerves (12), it has not been specifically demonstrated that leptin increases sympathetic outflow to WAT. Because physiological stimuli can promote sympathetic activity in a tissue-specific manner (44), it cannot be assumed that leptin causes a uniform increase in sympathetic activity of all peripheral tissues. Col-

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fat, it could potentially decrease fat depot size by antagonizing insulin, which would lead to a decrease in lipogenesis, by promoting lipolysis and by inhibiting preyhypo glycercer proliferation. The objective of the experiments described here was to determine whether sympathetic innervation is required for physiological doses of peripherally administered leptin to decrease body fat in mice and rats. This was achieved by infusing physiological doses of leptin into the infrapiritoneal cavity of mice or rats in which one fat pad had been sympathetically denervated by local injection of 6-hydroxycoccamadine (6OHDA). Theoretically, if the reduction in body fat caused by peripheral infusions of low doses of leptin involved activation of sympathetic outflow to WAT, then we would expect to see reductions in the size of intact white fat depots but not of the denervated fat pad in each leptin-treated animal. We chose to use 6OHDA because we wanted to produce a situation in which only one pad in each animal was denervated, allowing intact pads in the same animal to act as controls. Others have used either surgical denervation of white fat (9), which destroys both sensory and sympathetic nerves, or local injection of guanethidine, which produces selective sympathetic denervation of white fat (10). In preliminary studies, we did not find guanethidine to be effective in white fat of mice.

METHODS

Preliminary data: efficacy of sympathetic denervation by 6OHDA.

All animal procedures were approved by the Institutional Animal Care and Use Committee of the University of Georgia and were in accordance with the principles of the American Physiological Society (1). To confirm that 6OHDA decreased fat pad NE content, 13 5-wk-old C57BL/6 mice were obtained from Harlan (Indianapolis, IN) and were acclimated to housing (73°F with lights on 12 h/day from 7 AM) for 1 wk with free access to chow (Rodent Diet 5015; Lab diet, Purina Mills, MO) and water. The mice were anesthetized with isofluorane and then each mouse was injected 10 times with 2-µl injections of 8 mg/ml 6OHDA in 0.01 M PBS containing 1% ascorbic acid. Injections were given using a micro-syringe fitted with a 30-gauge needle. The needle was held in place for 45 s after each injection to minimize backflow. Injection of 6OHDA was alternated between sides of different mice to control for possible unilateral differences between pads. Six mice were killed 1 wk later and the remaining seven mice were killed after 2 wk. Both the injected and noninjected EPI pads were snap-frozen in liquid nitrogen for measurement of NE content, as described below.

Experiment 1: Effect of local denervation of one EPI pad on leptin response in C57BL/6 mice. Fifty-four 3-wk-old male C57BL/6 mice were single housed in conditions described above except that they were in cages with grid floors to allow measurement of food intake and were fed a diet containing 10% energy as fat (Diet 12450B, Research Diets, New Brunswick, NJ). After 1 wk of adaptation to the environment, baseline daily food intakes and body weights were recorded for 7 days and then the rats were divided into four weight-matched groups. The mice were anesthetized with ketamine (90 mg/kg) and a combination of xylazine (10 mg/kg) and glycopyrrolate (0.2 mg/ml). Each rat was fitted with an intraperitoneal Alzet miniosmotic pump (model 2002) delivering either 0.01 M PBS or 50 µg/day leptin (recombinant rat leptin: R&D Systems). At the same time, one EPI pad was injected 20 times with 2-µl injections of either 9 mg/ml 6OHDA in 0.01 M PBS, 1% ascorbic acid, or vehicle. The rats were housed as described above with free access to water and chow. Because of the large number of animals that were required, the study was completed using two cohorts of 40 rats with treatment groups equally represented in each cohort. Baseline daily measures of body weight and food intake were recorded for 7 days and then the rats were divided into 4 weight-matched groups of 10 animals per group. Each rat was anesthetized and one EPI fat pad, or one RP fat pad, was injected with multiple 2-µl volumes of either 6OHDA solution or vehicle, as described for experiment 2. EPI fat pads were each injected 20 times and RP pads were injected 10 times. The side of the rat on which the injected pad was located was randomized between animals. A miniosmotic pump (Alzet model 2002) was placed intraperitoneally and delivered either 50 µg/day leptin or an equal volume of PBS. Muscle and skin incisions were closed with sutures and the rats were given a subcu-
taneous injection of analgesic. The rats were allowed to recover consciousness before being returned to their home cages and were given a second injection of analgesic the day after surgery.

Daily measures of food intake and body weight continued for 13 days after surgery. Three days after surgery, the rats were food deprived from 7 AM to 12 PM and a small sample of blood was collected from the tail for measurement of serum leptin concentrations. On day 13 after surgery, rats were food deprived from 7 AM and were killed by decapitation between 9 AM and 12 PM. Individual white fat pads, IBAT, and left and right testes were dissected and weighed. The IBAT was snap-frozen and half of the pad was used for measurement of UCP1 mRNA expression by Northern blot and the other half was used to measure NE content. Portions of each EPI and each RP pad were snap-frozen for measurement of leptin mRNA expression by Northern blot or for determination of fat cell number and size distribution by Coulter counter. The average diameter of cells was calculated for each fat depot. The rest of the tissue, less the gastrointestinal tract, was returned to the carcass and body composition was calculated for each fat depot. The osmium tetroxide for determination of fat cell number and size distribution by Coulter counter. The average diameter of cells was calculated for each fat depot.

**NE measurements.** NE content of WAT and IBAT was measured by reverse-phase HPLC with electrochemical detection. EPI or RP fat was sonicated on ice (3× at 30 s) in 800 μl 0.2 M perchloric acid containing 3 μg/ml ascorbic acid and 25 ng/ml 3,4-dihydroxybenzylamine hydromide (DHBA) as an internal standard. The sonicated fat mixture was centrifuged for 15 min at 4°C at 9,000 rpm. After centrifugation, the infranate was removed and filtered through a 0.2-μm Nylon Sterile 25-mm syringe filter and 50 μl of the clear filtrate were injected into the HPLC system. IBAT was processed in an identical manner except that the sonication solution contained 100 ng/ml DHBA. NE was assayed using an ESA (Bedford, MA) HPLC system consisting of a model 582 Solvent Delivery Module, a model 542 auto sampler maintained at 6°C, and a model 5600A CoulArray detector at 350 mV. The column was a Phenomenex (150 × 4.6 mm) SYNERGI, 4 μ, Max RP-80A. The mobile phase consisted of 0.1 M sodium phosphate monobasic, 0.1 mM disodium EDTA, 0.3 mM 1-octanesulfonic acid, and 4% acetonitrile in ultra pure water. The pH of the mobile phase was adjusted to 3.1 with phosphoric acid. Chromatograms were analyzed with CoulArray for Windows, v.1.04, and NE content was calculated from a standard curve. Data are expressed as nanograms of NE per fat depot.

Statistical analysis. Body weight, food intake, and adipocyte size distribution were compared between groups using a repeated-measures ANOVA (Statistica Software 99th Edition, Stat Soft, Tulsa, OK). Body weight or food intake measured immediately after surgery was used as a covariate in the analysis. Fat pad weights, tissue NE content, serum leptin, FFA, triglycerides, and glycerol were compared using a two-way ANOVA. Independent variables in the analysis included 6OHDA vs. vehicle injections, leptin vs. PBS infusion, injected vs. noninjected pad, day for repeated measures of intake or body weight, cell diameter for measures of cell diameter, and EPI vs. RP denervation for experiment 3. Differences between specific groups were identified by post hoc Duncan’s multiple range test. The size of the denervated vs. intact EPI or RP fat pads within an animal was compared by paired t-tests. Differences were considered significant at P < 0.05. For reasons of clarity and simplicity, the detailed results of the statistical analysis have not been included except where necessary. References to significant differences indicate P < 0.05.

**RESULTS**

**Preliminary data.** Injection of 6OHDA significantly reduced NE content of mouse EPI fat 1 and 2 wk after injection by 47 and 43%, respectively (Fig. 1).

**Experiment 1.** Five days after surgery, 6OHDA/leptin mice had significantly higher serum leptin concentrations than all other groups and leptin was significantly higher in 6OHDA/PBS than vehicle/PBS mice (Fig. 2A). All of the mice lost weight in response to surgery but had recovered this weight by the second day of infusion. After this initial period of weight recovery, the 6OHDA/PBS mice gained significantly more weight during the experimental period than any other group (6OHDA/NS, Leptin: NS, 6OHDA × Leptin: P < 0.02: Table 1). This difference in weight occurred without any change in food intake (Table 1). At the end of the study, there was a small, but significant, reduction in the size of the noninjected EPI pads of vehicle/leptin mice, but this difference was not apparent in the injected pad. In contrast, leptin caused a significant reduction in the size of both the injected and noninjected EPI pads of 6OHDA/leptin mice (Fig. 2B). The noninjected pad was significantly larger than the injected pad in 6OHDA/PBS mice. Leptin did not produce significant changes in the size of RP or mesenteric fat depots in vehicle or 6OHDA-treated mice (data not shown). Surprisingly, with denervation of only one fat pad with 6OHDA, there was a significant increase in the total weight of dissected white fat from 6OHDA/PBS mice compared with all other groups (Table 1).

Leptin had different effects on total cell number of injected and noninjected EPI fat pads. There were significantly more cells in the injected pad of vehicle/leptin mice than in noninjected 6OHDA/leptin or noninjected vehicle/leptin pads (data not shown) and the difference in cell number was most obvious in the smallest diameter cells (data not shown). At the end of the study, there was no difference in NE content of any of the EPI pads and there were no differences in IBAT UCP1 mRNA expression or NE content (data not shown). There were no differences in serum triglycerides, glycerol, or FFA at the end of the experiment (data not shown).

**Experiment 2.** Neither leptin nor denervation of one EPI fat pad had a significant effect on food intake of the rats during the experimental period considered on either a daily basis or cumulatively over the 13 days of infusion (Table 2). All of the rats lost weight immediately after surgery and then regained the weight within 72 h. The 6OHDA/leptin rats weighed less...
vehicle or 6OHDA decreased the total number of cells present in the range of 50- to 80-μm diameter compared with the number of cells present in the contralateral, noninjected pad (data not shown), but there was no independent effect of 6OHDA or of leptin.

Surprisingly, denervation of one EPI fat depot had a significant effect on the leptin response of other fat depots within the same animal. The responses of RP and inguinal fat are represented as the total weight of the left plus the right pad. Leptin reduced the size of inguinal (Table 2) and RP fat depots (Table 2). The reduction was significant for vehicle/leptin but not 6OHDA/leptin rats. By contrast, leptin had no significant effect on the size of the mesenteric fat depot, but denervation of one EPI pad caused a significant increase in the amount of mesenteric fat present in 6OHDA/PBS rats (Table 2). There was no significant effect of either denervation or of leptin infusion on either the weight, the NE content, or the UCP1 mRNA expression of IBAT pads (data not shown). Despite the fat depot-specific effects of EPI denervation, there was no significant effect of denervation on carcass fat content of both vehicle/leptin and 6OHDA/leptin animals (Table 2).

Experiment 3. Leptin infusion caused a significant increase in serum leptin concentration (Fig. 4) measured 3 days after surgery. Leptin was significantly higher in the RP-denervated 6OHDA/leptin rats than in vehicle/leptin rats from either the RP denervation group or the EPI denervation group. Daily body weights and food intakes were not different between the

| Table 2. Food intake, body weight, and fat pad weights of rats in experiment 2 |
|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
|                             | Vehicle/ PBS | Vehicle/ Leptin | 6OHDA/ PBS | 6OHDA/ leptin |
| Cumulative food intake, g/13 days | 258±4 | 261±8 | 256±6 | 249±5 |
| Start weight, g | 252±4 | 256±2 | 252±2 | 253±3 |
| Weight gain, g/13 days | 58±2 | 57±3 | 53±3 | 52±3 |
| Inguinal fat, g/depot | 3.8±0.2A | 3.0±0.2B | 3.8±0.3A | 3.3±0.3AB |
| Mesenteric fat, g/depot | 1.8±0.1A | 1.6±0.1A | 2.4±0.1B | 2.0±0.02AB |
| RP fat, g/depot | 0.37±0.03A | 0.22±0.04A | 0.45±0.06A | 0.30±0.03A |
| Carcass fat, g/rat | 31±2A | 26±2B | 31±2A | 24±2B |

Data are means ± SE for groups of 10 rats per group in experiment 2. Start weight indicates the weight of the rats on the morning of the surgery. Values for fat pads and carcass fat that do not share a common superscript are significantly different at P < 0.05. RP, retroperitoneal.
eight treatment groups during the baseline period (data not shown). The surgery caused a transient inhibition of food intake in all rats that was reversed within 2 days in the PBS-infused rats but was slower to recover in leptin-infused rats. There was no effect of 6OHDA on food intake of EPI-denervated rats, but in the RP-denervated group 6OHDA/leptin rats took 1 extra day for their food intake to reach control levels than did the vehicle/leptin rats (data not shown). When total food consumed during the 13 days of infusion was considered, 6OHDA/leptin rats ate significantly less than 6OHDA/PBS rats in the RP denervation group (Table 3). Consistent with this difference in food intake, 6OHDA/leptin rats gained less weight during the 13 days of leptin infusion than vehicle/leptin rats in the RP denervation group (EPI vs. RP: NS, 6OHDA: NS, Leptin: \( P < 0.03 \), Leptin \( \times \) 6OHDA: \( P < 0.05 \); Table 3).

Neither leptin nor denervation produced any significant change in the NE content of either EPI or RP fat pads from EPI-denervated rats at the end of the experiment (Fig. 5, top and bottom left). In contrast, denervated RP pads from RP-denervated rats had less NE than the contralateral, noninjected pads within the same animal (Fig. 5, bottom right). The NE content of the EPI pad in RP-denervated rats that was contralateral to the denervated RP pad was significantly elevated (Fig. 5, top right). NE concentration, expressed as nanograms per gram of tissue, was substantially lower in noninjected EPI than RP pads (48 ± 5 vs. 101 ± 9 ng/g tissue), but because the EPI pads were larger, total NE per fat pad was greater in EPI than RP pads.

There was no effect of leptin or of either EPI or RP denervation on the weight of EPI fat depots (data not shown). In addition, there were no differences in the weights of injected vs. noninjected (contralateral) pads, indicating that the multiple 2-μl injections had not caused any significant physical damage to the tissues, although this did not exclude the possibility of subtle changes in metabolism or responsiveness of the cells. In

Table 3. Food intakes and body weights of rats in experiment 3

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Start Weight, g</th>
<th>End Weight, g</th>
<th>Weight Gain, g/13 days</th>
<th>Food Intake, g/13 days</th>
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<td>EPI Denervated</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle/PBS</td>
<td>273 ± 5</td>
<td>309 ± 9</td>
<td>36 ± 5 (^{AB})</td>
<td>297 ± 13 (^{AB})</td>
</tr>
<tr>
<td>Vehicle/leptin</td>
<td>273 ± 5</td>
<td>307 ± 7</td>
<td>34 ± 3 (^{AB})</td>
<td>290 ± 8 (^{AB})</td>
</tr>
<tr>
<td>6OHDA/PBS</td>
<td>275 ± 2</td>
<td>311 ± 5</td>
<td>37 ± 2 (^{AB})</td>
<td>299 ± 5 (^{AB})</td>
</tr>
<tr>
<td>6OHDA/leptin</td>
<td>272 ± 4</td>
<td>301 ± 6</td>
<td>30 ± 3 (^{AB})</td>
<td>281 ± 6 (^{AB})</td>
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<tr>
<td>RP Denervated</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle/PBS</td>
<td>273 ± 5</td>
<td>308 ± 7</td>
<td>35 ± 3 (^{AB})</td>
<td>296 ± 8 (^{AB})</td>
</tr>
<tr>
<td>Vehicle/leptin</td>
<td>274 ± 3</td>
<td>309 ± 4</td>
<td>35 ± 3 (^{AB})</td>
<td>285 ± 6 (^{AB})</td>
</tr>
<tr>
<td>6OHDA/PBS</td>
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<td>309 ± 9</td>
<td>39 ± 0 (^{A})</td>
<td>304 ± 10 (^{A})</td>
</tr>
<tr>
<td>6OHDA/leptin</td>
<td>273 ± 6</td>
<td>299 ± 10</td>
<td>27 ± 6 (^{B})</td>
<td>280 ± 7 (^{B})</td>
</tr>
</tbody>
</table>

Data are means ± SE for groups of 10 rats. Start weight was the weight of the animals the morning of surgery (day 0) and end weight was the weight of the rats on day 13 of infusion. Values for weight gain or food intake that do not share a common superscript are significantly different at \( P < 0.05 \). EPI, epididymal. RP, retroperitoneal.
contrast, leptin reduced RP fat pad weights of rats in both EPI- and RP-denervated groups (Fig. 6A), although there was no significant effect of denervation on the size of RP pads. Neither denervation nor leptin changed cell number in either RP or EPI fat pads of the RP-denervated groups (data not shown). Denervation of one EPI fat pad did not change the number of cells present in the injected, denervated pad and leptin infusion had no effect on EPI cell number in any treatment group. Surprisingly, denervation of one EPI pad caused a significant increase in cell number of the noninjected, contralateral EPI pad and the contralateral RP pad of 6OHDA/PBS rats, but this hyperplasia was blocked in the 6OHDA/leptin rats (Fig. 7, C and D). The cell size distribution showed that the increase in cell number was represented in all cell sizes up to 90-μm diameter (Fig. 7, A and B) and there was no change in the average diameter of cells in either EPI or RP pads from PBS-infused rats, although leptin reduced the average cell diameter in RP pads from all treatments (P < 0.0001; Fig. 7, E and F). Leptin significantly reduced the size of inguinal fat pads but there was no effect of denervation on inguinal size or leptin response (data not shown). Leptin also reduced the size of mesenteric fat pads in the vehicle/leptin rats in the RP-denervated group but did not change mesenteric pad size in the EPI-denervated group (Fig. 6B). At the end of the study, carcass fat content, expressed either as grams per rat or as percent carcass weight, was significantly reduced by leptin in the EPI-denervated group (Fig. 6C), but there were no differences in lean tissue mass. The effect of leptin did not reach significance in the RP-denervated group due to the carcass fat content of the control, vehicle/PBS, and 6OHDA/PBS rats being lower in the RP-denervated group than in the EPI-denervated group. There was no effect of either denervation or of leptin infusion on IBAT UCP1 mRNA expression or NE content. Denervation had no effect on EPI pad leptin mRNA expression. There was a trend for leptin to inhibit expression but this did not reach statistical significance (P < 0.08; data not shown).

DISCUSSION

In the studies reported here, we produced a selective sympathetic denervation of fat pads by making local injections of 6OHDRA into the pad and thereby destroying its sympathetic innervation (40). The results from NE content measures at the end of the experiments, 13 days after the denervation, indicated

![Epi NE Content](image1)

![RP NE Content](image2)

Fig. 5. NE content of EPI pads in EPI-denervated (top left) and RP-denervated (top right) rats and of RP pads in EPI-denervated (bottom left) and RP-denervated (bottom right) rats. Data are means ± SE for groups of 10 rats. Values on a given axis that do not share a common superscript are significantly different at P < 0.05.
insignificant reductions in NE content in EPI pads but a more robust (40–50%) reduction in RP pads. This decrement is similar to that found in 6OHDA-injected white fat in Siberian hamsters (Song CK and Bartness TJ, unpublished data). We attribute the difference in response between EPI and RP depots to the difference in NE concentration in the two pads. The amount of NE per milligram of tissue in EPI fat is much lower than that in RP fat; therefore, it may have been difficult to identify denervations in this tissue. Although decreases in EPI NE content were not measurable at the end of the experiment, the sympathetic denervation had to have been at least partially effective, otherwise we would not have been able to differentiate between the denervated animals and those that were injected with vehicle. This appears to be the first report of using 6OHDA to produce a tissue-specific denervation in white fat and we patterned our methodology after that of Mayerhofer et al. (31), who made local injections of 6OHDA in testes and found undetectable testicular NE content 24 h after the injection (NE concentration was not measured at any subsequent time points). In experiments involving whole animal sympathetic denervation, 6OHDA treatment reduced the NE content of IBAT to 10–35% of control values 2 days after injection but these levels doubled over the next 6 days (11). In other studies that specifically denervated WAT, total surgical denervation produced a dramatic and sustained reduction in tissue NE content (9), but we wanted a selective sympathetic denervation and surgical denervation does not discriminate between sympathetic and sensory nerves. More recently, Demas and Bart-

Fig. 6. Weights of individual RP pads (A) in EPI-denervated and RP-denervated rats and of mesenteric fat pads (B) in EPI-denervated and RP-denervated rats and percent carcass fat measured at the end of the experiment (C). Data are means ± SE for groups of 10 rats. Values on a given axis that do not share a common superscript are significantly different at P < 0.05.
ness (10) reported an effective sympathetic denervation in hamster white fat using localized injections of guanethidine, but we were unable to produce equivalent effects in mouse adipose tissue with this chemical.

The primary objective of these studies was to determine whether peripherally infused physiological concentrations of leptin required activation of the SNS to cause a selective reduction in body fat mass. Others reported that innervation is not required for leptin to reduce the size of fat transplanted into rats in which hyperleptinemia was induced by adenovirus (43). The circulating concentrations of leptin found in adenovirus-treated rats (23/1100612 ng/ml) were much greater than those found in normal weight rats and caused a significant inhibition of food intake. In this study, however, we determined whether physiological levels of leptin that did not inhibit food intake required SNS activity to reduce fat depot size. In the first two experiments, EPI fat was denervated because previous experiments showed this pad to be responsive to peripheral infusions of leptin (4, 35) and because it is easy to inject the pad uniformly with minimal trauma to the animal. In experiment 1 with mice, leptin decreased the mass of denervated EPI fat to the same degree as depots with intact sympathetic nerves. In experiment 2 with rats, however, leptin had little effect on EPI weight and it was difficult to conclude whether sympathetic innervation was an important mediator of leptin action in rats. Specifically, although we did not find a significant leptin effect in either the 6OHDA- or vehicle-treated fat depots, denervating one EPI pad led to increased mesenteric fat pad weight and reduced the leptin response in intact RP and inguinal fat pads in the same animal. These results demonstrate that the denervation was effective even though it may have been transient and/or incomplete. Similar results were obtained with EPI denervation in experiment 3, but in this experiment the results from RP-denervated animals clearly show that sympathetic denervation does not prevent leptin from reducing fat pad mass. The activation of the SNS by leptin (25) and the

Fig. 7. Fat cell size distribution in EPI pads (A) and RP pads (B) for EPI-denervated rats and total fat cell number for the same fat pads (C and D). E and F: average cell diameter for the EPI and RP fat from EPI-denervated rats. There was a significant effect of leptin (P < 0.0001) on the average size of cells in the RP pads but there were no significant effects for any specific treatment group. Values for total cell number for EPI or for RP pads that do not share a common superscript are significantly different at P < 0.05. Data are means ± SE for groups of 10 rats.
inhibition of adipose leptin mRNA expression by adrenergic agonists (20) strongly suggest that these two factors interact to regulate body fat mass. The results from experiments 1 and 3, however, support the view that peripherally infused leptin does not require activation of the SNS to reduce the size of white fat depots.

Although we did not explore the mechanisms responsible for the reduction in fat pad size of leptin-treated animals in these experiments, measurements of fat cell size and number suggest that leptin causes a loss of lipid from the cells, rather than causing a dramatic decrease in fat cell number. This suggests that the majority of the loss of fat was due to changes in adipocyte metabolism, such as increased lipolysis (15) and/or decreased lipogenesis (21), rather than a significant stimulation of apoptosis or an inhibition of preadipocyte differentiation. A second piece of evidence supporting the independence of leptin from the SNS was that although we expected that denervation would decrease the responsiveness to leptin, we found that denervation of one fat depot exaggerated the effects of leptin on body weight and fat pad size in mice in experiment 1 and on body weight of rats in experiment 3. This argues against the SNS being essential for leptin action on body weight and body fat and also indicates that denervation of a single fat depot may influence the whole animal response to leptin.

The leptin infusions used in the studies described here caused relatively small increases in serum leptin concentration, reinforcing the fact that the doses given resulted in circulating concentrations that were within the physiological range. Surprisingly, denervation of one fat pad in both PBS- and leptin-infused mice increased circulating leptin concentrations. This is consistent with the observation that activation of the SNS inhibits WAT leptin mRNA expression (8) and with observations that spinal cord injury patients lacking a functional sympathetic chain show increased circulating serum leptin concentrations (27). Although denervating one pad might not be expected to change circulating leptin levels, if denervation of one pad suppressed SNS activity in other, intact depots, as discussed below, then there could be a significant increase in circulating concentrations of leptin. Denervation alone did not increase circulating concentrations of leptin in rats and we found no significant effect of denervation on leptin mRNA expression in EPI fat in experiment 3. These results suggest either species differences in the regulation of leptin production or that the conditions of this study were too subtle to produce measurable changes in leptin expression in rats.

In the experiments described here, we gave the rats and mice peripheral infusions of physiological doses of leptin. Previous studies showed that these doses effectively reduce body fat (21, 23) even though they are lower than those used by many other investigators. Evidence is available for both centrally administered leptin and for higher peripheral doses of leptin to increase sympathetic output to tissues other than white fat (8, 25, 39). Here, we found that low doses of peripherally administered leptin increased NE content in the noninjected EPI pads of vehicle/leptin mice and rats. The increase in NE content was small but consistent with results reported by Collins et al. (7) where peripheral injections of leptin caused a nonsignificant increase in the NE turnover in RP fat of ob/ob mice. Increased NE content is frequently associated with increased NE turnover (45). The failure of leptin to increase NE content in the vehicle-injected fat depots suggests that the surgery caused some disruption of the pads even though there were no significant effects on fat cell number or size. Others have reported that exposure and mechanical stimulation of white fat cause a reduction in tissue NE content to a level intermediate between denervated and untouched fat depots (45). Thus, although we did minimize manipulation of the pads and used 30-gauge needles for injections, there may have been some trauma that made them less responsive to leptin. There was no evidence for increased NE content in brown adipose tissue or UCPI mRNA expression in any leptin-treated rats or mice in the experiments described here. Others have shown that central and peripheral administration of leptin activates brown fat thermogenesis (8, 42); therefore, it is possible that the amount of leptin used in this study was too low to activate thermogenesis or that the small change in energy expenditure that would be required to produce the observed loss of fat did not require measurable changes in UCPI mRNA expression.

In experiment 2, we were surprised to find that denervation of one EPI pad changed the leptin responsiveness of other intact white fat depots. This effect was also apparent in the combined weight of inguinal fat pads in EPI-denervated rats, but not RP-denervated rats, in experiment 3 (data not shown) and we found an increase in the size of the noninjected EPI pad of 6OHDA/PBS mice in experiment 1. Thus destruction of sympathetic nerves in one fat pad influenced distant but neurally intact fat pads. Assuming that this response was specific to EPI denervation implies a complex interaction between fat pads that may be influenced by the level of SNS input to the denervated pad and the connections and functions of its sensory innervation. Intact EPI fat had a lower NE concentration, per unit weight, than RP fat and presumably this indicates a lower level of SNS innervation or drive, which would have to be confirmed by measures of NE turnover and by histological evaluation of innervation. Another factor to consider is that EPI fat is integral to the support of reproductive function (38), and it is possible that it is loss of control of some aspect of this important physiological function, rather than level of SNS innervation or drive, that determines the impact of EPI vs. RP denervation. Although the results of experiments 1 and 2 did not differentiate between humoral and neural communication between the denervated and intact pads, the results from experiment 3 suggest that the response in distant pads was mediated by neural connections. Specifically, denervation of one EPI pad caused significant increases in fat cell number only in the RP and EPI pads contralateral to the denervated EPI pad and not in the ipsilateral RP pad. A uniform response would be expected in both RP pads if the effect was mediated by a circulating factor, assuming uniform expression of receptors and postreceptor signaling proteins in the two RP pads. Because the enlargement of contralateral fat pads was most striking in denervated rats infused with PBS, it appears that leptin directly opposed the effect that was initiated by selective denervation, but it is not clear whether the two factors acted on identical metabolic pathways or simply in opposition to one another. It also is not clear from the results of these experiments whether the blunted leptin response in intact pads of EPI 6OHDA/leptin rats in experiments 2 and 3 was due to a change in leptin responsiveness or to opposing, but independent, effects of leptin and the disruption of neural input on fat cell metabolism. These issues need to be resolved in experiments that are specifically designed to investigate the effects of...
denervation. In this study, our primary objective was to examine the effects of leptin on denervated fat pads; therefore, the experimental design did not allow us to evaluate many aspects of the response of intact pads in the denervated rats and mice. Because local injections of 6-OHDA would not cause global denervation (33), it would appear that there is some communication between pads resulting in changes in SNS drive. The mechanisms by which this might occur are unknown but may involve sensory innervation of white fat (14, 36) if the regulation of specific fat depots is mediated by sensory afferent fibers responding to metabolic or cellular changes that are controlled by sympathetic efferent fibers. Niijima (32) previously reported that injecting leptin into one EPI fat pad increases sympathetic activity in the contralateral pad, supporting the concept of neural communication between different fat depots. In the studies described here, denervating one fat depot did change the size of other fat pads in rats and total body fat mass of mice in experiment 1, but the effects were not large. These responses may have been greater if the study had been extended, but because the focus of this study was the effect of leptin on fat pad size, the experiment lasted for only 13 days. Demas and Bartness (10) found that chemical sympathectomy of inguinal fat in hamsters caused a doubling of size of the denervated pad in a 2-wk period. The differences between the outcome of that study and the experiments described here may be due to differences in species used or the fat depot that was denervated. Unlike previous reports for surgical denervation of rat (9) or hamster WAT (5, 45), we did not find any significant increase in fat cell number in the denervated fat pads of rats or mice. It is possible that because the rats and mice in this study were young and were already growing rapidly, there was a ceiling effect on preadipocyte proliferation. The results of experiment 3, however, argue against this because we found a significant hyperplasia in the contralateral EPI and RP fat pads of EPI-denervated rats. Leptin infusion prevented this increase in cell number, implying that the SNS normally integrates information across fat depots and controls cell number accordingly and that leptin is capable of controlling cell number through similar mechanisms. Because we did not find a significant effect of leptin on cell number in intact fat depots, it is possible that a maximal level of inhibition of proliferation is achieved with normal sympathetic tone and that leptin does not produce an additive effect.

In summary, the results from these experiments suggest that SNS outflow into white fat is not essential for leptin to reduce fat mass in mice or rats. Because we used low concentrations of peripherally administered leptin, it is possible that opposite effects or more marked effects may be seen if leptin is administered centrally or if larger doses of leptin were administered peripherally, resulting in nonphysiological circulating concentrations of the protein. Surprisingly, denervation of one fat depot caused significant changes in neurally intact, distal fat pads in both rats and mice. Therefore, it is likely that some signal is communicating from the denervated pad leading to changes in other fat depots. Whether this signal is a circulating factor or a neural signal is unknown, but the results from experiment 3 support the concept of neural communication. Some evidence for direct neural communication between fat depots comes from the observations that the injection of leptin into one EPI pad increases efferent sympathetic flow into the contralateral pad (32). Further studies are needed to determine exactly which metabolic pathways are modified by leptin to reduce body fat mass and whether these changes are induced indirectly or by leptin acting directly on white fat. In addition, studies should be conducted to identify the nature of the signal between fat depot that facilitates an orchestrated response to sympathetic denervation of a single fat pad.

GRANTS

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