Differential Sensitivity to Central Leptin and Insulin in Male and Female Rats
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The distribution of fat in the body differs between the male and female sexes and is associated with the relative secretion of the two “adiposity” hormones leptin and insulin. We now report that the brains of male and female rats are differentially sensitive to the catabolic actions of small doses of these two hormones. Leptin (1 or 3.5 µg/2 µl) or saline (2 µl) was administered into the third cerebral ventricle of age- and weight-matched male and female rats. Leptin significantly reduced food intake in female and male rats over 4 h; however, leptin reduced 24-h intake in female but not in male rats. When the same rats were administered insulin (1 or 4 µU/2 µl) or saline (2 µl), male but not female rats had a robust reduction in food intake over 24 h. Previous research demonstrates the melanocortins are a central mediator of the effects of both leptin and insulin. However, we found no sex differences in sensitivity to the melanocortin agonist MTII (0.01, 0.1, 0.3, and 1.0 nmol/2 µl). These results suggest that the sex differences in sensitivity to leptin and insulin at the doses that we injected occur upstream of the melanocortin receptors. Because insulin and leptin reflect different fat beds and are differentially distributed in the male and female sexes, the implication is that the male and female sexes regulate adiposity-relevant parameters differently. Diabetes 52:682–687, 2003

The increasing prevalence of obesity throughout the world is associated with an escalating incidence of obesity-related disorders and health costs (1,2). One of the more important discoveries in recent years was the finding that the distribution of fat within the body is associated with the risk for developing obesity-related complications (3). Fat distributed in the abdominal or visceral region carries a much greater risk for cardiovascular problems, type 2 diabetes, certain cancers, and other disorders than does fat distributed subcutaneously (3,4). Of particular importance to public health is that the distribution of fat varies greatly between men and women. On average, women carry more fat subcutaneously, whereas men carry more fat viscerally (3). Hence, there are major sex differences with regard to obesity-associated health risks, men being more likely to develop cardiovascular problems, diabetes, and other obesity-related problems (5). Because of this, it is vitally important to determine whether there are parallel differences in the way men and women detect and respond to signals that control energy balance and metabolism.

Hormones secreted in proportion to body fat provide an important regulatory signal to the brain. In particular, leptin and insulin are each secreted into the plasma in direct proportion to body fat, and each peptide is transported into the brain (6,7), where it acts on specific receptors on neurons in the hypothalamus and other brain areas (8,9). Increased activity of either peptide locally in the vicinity of the ventral hypothalamus causes an overall catabolic response (i.e., reduced food intake, increased energy expenditure, and loss of body weight), whereas decreased activity of either hormone in the same sites causes an overall anabolic response (i.e., increased food intake, decreased energy expenditure, and increased body weight) (8–10). Insulin and leptin share many common functions regarding the control of energy homeostasis, and recent evidence suggests that they also share common intracellular signaling pathways (8,11).

Insulin secretion is highly correlated with visceral fat content as well as with the risk for developing complications of obesity (12), whereas leptin secretion correlates better with subcutaneous fat and is therefore less of a risk factor for complications of obesity (13). Hence, insulin is a better predictor of total body fat in men and leptin is a better predictor of total body fat in women (3,13). Consistent with these findings, leptin levels are directly correlated with estrogen levels in women (14,15); estrogen reduces food intake and body weight in women (16). In addition, leptin levels are significantly higher in female than in male rats, when equated for body weight (17,18). Leptin levels have not been reported to be influenced by the estrous cycle (17) but are decreased by ovariectomy (19,20). On the basis of this evidence, we hypothesized that central leptin would be relatively less efficacious in reducing food intake in female rats and that, conversely, central insulin would be relatively less efficacious in male rats. An important implication is that the integrated “adiposity” message conveyed to the brain differs in male and female sexes.

RESEARCH DESIGN AND METHODS
Animals. Adult male and both age- and weight-matched female Long-Evans rats (Harlan, IN) were used. Two groups of female rats were included because age-matched female rats weigh less than male rats after puberty. Male rats in

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ARC, arcuate nuclei; i3vt, third-cerebral ventricle; POMC, proopiomelanocorticotropin.
each experiment were 65–70 days old and weighed 250–274 g. age-matched female rats were 65–70 days old and weighed 200–224 g; and weight-matched female rats were 111 days old and weighed 250–374 g. Animals were individually housed in Plexiglas tubes and maintained on a 12:12-h light-dark cycle in a temperature-controlled, AAALAC-accredited vivarium, and all procedures were approved by the Internal Animal Care and Use Committee at the University of Cincinnati. The rats were maintained on ad libitum pelleted food and tap water unless otherwise noted. Seven days after arrival in the laboratory, rats were anesthetized with 1.0 ml/kg ketamine/xylazine (10:6.5 ratio), and 21-gauge stainless-steel guide cannulas were implanted (Plastics One, Roanoke, VA) in the skull with the tip aimed at the third-cerebral ventricle (i3vt). Bregma and lambda were positioned at the same vertical coordinate, and the sagittal sinus was carefully displaced laterally with a metal probe as the guide cannula was lowered directly on the midline, 2.2 mm posterior to bregma, to a point 7.5 mm ventral to dura. Guide cannulas were fixed to the skull with anchor screws and dental acrylic. The guide cannulas were fitted with removable obturators that extended 0.5 mm beyond the tip (21). When rats regained their preoperative body weights after surgery, placement of i3vt cannulas was confirmed by administration of 10 ng of angiotensin II in 1 μl of normal saline while the animals were water replete. Animals that did not drink at least 5 ml of water within 60 min were not used.

**Third-ventricular administrations.** Male and age- and weight-matched female rats (experiment 1) had their food removed 4 h before the onset of the dark and were given a bolus i3vt injection of one of two doses of insulin (Iletin II Regular pork insulin; Eli Lilly, Indianapolis, IN; 1 or 4 mU/2 μl) or vehicle (saline, 2 μl) at that time. All rats received the three injections in a counterbalanced design, subsequent injections occurring after complete recovery of food intake and body weight to baseline levels, generally 5 days after the first treatment. Food was returned at the onset of dark, and intake was measured over the subsequent 1, 4, and 24 h (for brevity, we have presented the data for only the 4- and 24-h time points; the findings are comparable for the 1- and 4-h food intakes). The same rats were then administered leptin (1.0 or 3.5 μg/2 μl Human Leptin; CalBiochem, San Diego, CA) or vehicle (saline, 2 μl) in the same paradigm on separate days in random order (experiment 2). In experiment 3A, novel cohorts of rats received one of six injections: saline or 0.01, 0.03, 0.1, 0.3, or 1.0 nmol MTII (American Peptide Company, Sunnyvale, CA), a mixed melanocortin 3/4 receptor agonist, in 2 μl of saline on separate days in random order. At the end of this series of injections, these rats were given a bolus injection of leptin (3.5 μg/2 μl) or vehicle (saline, 2 μl) on separate days in random order (experiment 3B).

**Plasma analysis.** Separate cohorts of female and weight-matched male rats were fasted overnight and killed by decapitation. Trunk blood was collected, and the plasma was isolated by centrifugation and stored at −80°C until analyzed by radioimmunoassay for plasma leptin using a rat leptin radioimmunoassay kit (Linco Research, St. Charles, MO).

**RESULTS**

Two groups of female rats were used in all experiments, one having the same age and the other the same initial body weight as a cohort of male rats. Although there were no reliable differences between the two groups of female rats on any dependent variable in any experiment, the two groups of female rats were analyzed separately in one analysis and combined in an independent analysis. The conclusions from both analyses were identical.

**I3vt insulin reduces food intake and body weight of male but not female rats (experiment 1).** Male rats were significantly more anorexic than either group of female rats after 4 mU of i3vt insulin (Fig. 1A). In addition, the weight loss in the male rats achieved significance during the 24 h after the injections (−8.3 ± 3.5 g; P < 0.05). The 24-h change in body weight was not significant for either group of female rats (age-matched −7.6 ± 2.6 g [NS] and weight-matched −2.9 ± 2.1 g [NS]). The male rats also reduced their food intake after a lower dose of insulin (1 mU/2 μl), whereas there was no effect in the female rats (Fig. 1B). Again, male rats lost a significant amount of body weight after the 1-mU insulin injection (−4.25 ± 0.9 g; P < 0.05), whereas the 24-h change was not significant in either the age-matched (3.2 ± 1.0 g; NS) or the weight-matched female rats (1.8 ± 0.8 g; NS).
I3vt leptin reduces food intake and body weight to a greater extent in female than male rats (experiment 2). I3vt leptin (3.5 μg/2 μl saline) reduced food intake comparably in both sexes after 4 h (Fig. 2A). This anorexia persisted in both groups of female rats, remaining significant after 24 h (Fig. 2A). In contrast, the anorexic effect of the leptin was short-lived and no longer apparent by 24 h in the male rats (Fig. 2A). Both groups of female but not the male rats also lost significant body weight during the 24 h after i3vt leptin (age-matched female rats −7.6 ± 2.7 g [P < 0.05], weight-matched female rats −10.0 ± 2.2 g [P < 0.05], male rats −2.0 ± 1.7 g [NS]). Male and female rats reduced their food intake 4 h after an injection of a lower dose of leptin (1 μg/2 μl saline). Once again, this anorexia persisted in female but not male rats. Twenty-four hour food intake was significantly reduced by the lower dose of leptin in female rats. Twenty-four hour intake was not significantly reduced in male rats (Fig. 2B). Both cohorts of female rats also had a significant reduction in body weight (age-matched female rats −4.9 ± 0.8 g [P < 0.05], weight-matched female rats −5.2 ± 1.1 g [P < 0.05]), whereas the male rats (1.9 ± 2.2 g; NS) did not.

I3vt MTII reduces food intake comparably in female and male rats (experiment 3A). As depicted in Fig. 3A, i3vt MTII (0.01, 0.03, 0.1, 0.3, 1.0/2 μl of saline) reduced food intake dose-dependently and comparably in both sexes after 4 and 24 h (4-h data not shown). All three groups also lost significant and comparable body weight during the 24 h after the largest dose of i3vt MTII (data not shown).

I3vt leptin reduces food intake and body weight of female but not male rats (experiment 3B). To confirm that leptin reduces food intake and body weight differentially in male and female rats, the same animals (experiment 3A) received an injection of leptin at a dose of 3.5 μg/2 μl saline. Consistent with experiment 2, the female rats were relatively more sensitive to leptin (Fig. 3B). In addition, both groups of female rats lost significant weight 24 h after leptin (age-matched female rats −12.5 ± 4.7 g [P < 0.05], weight-matched female rats −13.6 ± 5.4 g [P < 0.05]), whereas the male rats did not (−2.1 ± 2.2 g; NS).

Plasma leptin (experiment 4). Weight-matched female rats had significantly higher plasma leptin levels than male rats (P < 0.05; Fig. 4A). These findings are consistent with previous reports for rats (17) and humans (3,13).

Body composition (experiment 5). Female rats also had significantly more carcass fat than the male rats (P < 0.05; Fig. 4B) and significantly more leptin per gram of body fat (P < 0.05; Fig. 4C).

DISCUSSION

We have found that the brains of female rats are relatively more sensitive to the catabolic actions of leptin, whereas the brains of male rats are more sensitive to the catabolic actions of insulin at the doses given. However, we found no sex differences in sensitiveness to a melanocortin agonist, even though the melanocortins are known to mediate some of the effects of both leptin and insulin (22). An important implication of these findings is that the "adiposity" message conveyed to the brain differs in male and female rats. Male rats are relatively more sensitive to the central catabolic actions of insulin, demonstrated by a significant reduction in 24-h food intake after the doses that we used. In contrast, the female rats are relatively more sensitive to the central catabolic actions of leptin after the doses that we used as manifested by a greater reduction of food intake and body weight. In addition, we found that female rats have more body fat and higher
plasma leptin levels per gram of fat, consistent with previous reports (17,18) and consistent with sex differences in the levels of reported circulating leptin in humans.

We interpret our results to suggest that the differential sensitivities that we have observed are not absolute. Numerous experiments have reported that male rats are sensitive to the central administration of leptin (23). What is novel in the present experiments is that when the dose of leptin is low and held constant and male and female rats are precisely matched, female rats exhibit significant reductions in food intake and body weight at lower doses of leptin than male rats. The male rats in the present experiments in fact had a significant reduction of food intake after the larger dose of leptin, but it was short-lived, relative to the response in the female rats. It may also be the case that female rats are sensitive to some doses of centrally administered insulin, but this has not been rigorously tested. In one experiment, female rats were administered insulin i3vt at doses ranging from 0.5 to 10 mU/day, with no reliable change of food intake or body weight (24). Whether higher doses of insulin would be efficacious in female rats is not known.

Insulin secretion is highly correlated with the amount of visceral fat as well as with the risk for developing complications of obesity (12,25), whereas leptin secretion correlates better with subcutaneous fat (13,26). Male rats have predominantly more visceral adiposity, which is correlated with plasma insulin levels and is therefore a better predictor of total body fat in male rats. The converse is true, with subcutaneous fat being highly correlated with plasma leptin levels and therefore being a better predictor of total body fat in female rats (3,13). Contrary to our initial hypothesis, female rats were more sensitive to the central administration of leptin, despite having higher plasma leptin. Our findings do not identify the mechanism underlying this enhanced sensitivity to centrally administered leptin. However, there are several possible explanations that can be addressed in future work. For example, there may be differences in leptin transport across the blood-brain barrier or differences in expression levels of the long form of the leptin receptor between weight-matched male and female rats. The same rats in A were subsequently administered leptin (3.5 μg/2 μl saline) or saline alone (2 μl) i3vt. Repeated measures ANOVA revealed that leptin significantly reduced intake 4 and 24 h in female but not in male rats. Bars are means ± SE; *P < 0.05.
and female rats and observed no difference in sensitivity, suggesting that the basis of the sex difference in sensitivity to leptin and insulin must occur upstream of the melanocortin receptors or in another potential mediator of leptin and insulin signaling, neuropeptide-Y (NPY).

It is possible that receptors for gonadal steroids on POMC cells underlie the sex difference. Estrogen normally acts in the brain to reduce food intake and body weight in female individuals (35–40). Although the basis of the interaction between estrogen and leptin is not known, there are reports that leptin receptors and estrogen receptors are colocalized on the same hypothalamic neurons (41,42), suggesting that one point of interaction may be in individual hypothalamic neurons. Whatever the mechanism, one implication of these sex differences is in the development of therapeutic approaches for treating obesity in men and women. Furthermore, there may well be sex differences in how the brain influences the susceptibility to the health risks that accompany visceral adiposity.

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