Differential role of melanocortins in mediating leptin’s central effects on feeding and reproduction

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Am. J. Physiol. Regulatory Integrative Comp. Physiol. 278: R50–R59, 2000.—Leptin serves as a humoral link coupling the status of energy reserves to the functional activity of the reproductive system. Leptin is thought to act through melanocortinergic pathways in the brain to regulate ingestive behaviors; however, whether melanocortins mediate leptin’s actions on the neuroendocrine-reproductive axis is unknown. We tested this hypothesis first by determining whether the effects of leptin on feeding behavior and reproduction in the ob/ob mouse could be blocked by the melanocortin receptor (MC-R) antagonist SHU9119 and second, by examining the effects of the MC-R agonist MTII on feeding and the endocrine-reproductive system. Administered by intracerebroventricular injections, leptin inhibited food intake, raised plasma gonadotropin levels, and increased seminal vesicle weights compared with controls; SHU9119 (intracerebroventricularly) attenuated leptin’s effects on food intake and body weight but did not alter leptin’s stimulatory effect on the reproductive axis. MTII (intracerebroventricularly and intraperitoneally) decreased food intake and increased body temperature compared with controls but had no effect on the reproductive-endocrine axis. These results suggest that although leptin acts centrally through melanocortinergic pathways to inhibit ingestive behaviors and stimulate metabolism, leptin’s activation effect on the reproductive axis is likely to be mediated by other, unknown neuroendocrine circuits.

LEPTIN IS AN ADIPOCYTE-DERIVED hormone that plays a key role in the regulation of body weight and reproduction (1, 60). In rodents, the absence of leptin or defects in the leptin receptor result in obesity and infertility (8, 28). Likewise, defects in the leptin or leptin receptor genes in humans cause states of morbid obesity and hypogonadism (11, 40). The administration of leptin to leptin-deficient ob/ob mice inhibits feeding, reduces body weight, and restores fertility (2, 4, 7, 42). Some of leptin’s biological effects may reflect its actions on peripheral target sites (29, 52); however, leptin also acts on the brain to influence the circuitry governing ingestive behavior, thermogenesis, and reproduction (14, 21, 27, 32). Although some of the target cells for leptin’s action have been identified (9, 20, 38), we are only beginning to elucidate the role of particular neurotransmitters and their receptors in mediating the action of leptin on the brain, and we do not know yet whether the effects of leptin on feeding and reproduction are mediated by shared or separate circuits in the nervous system.

Several hypothalamic neuropeptides have emerged as prime targets for leptin’s action on the brain. Included among these is α-melanocyte-stimulating hormone (α-MSH), a melanocortin cleavage product of proopiomelanocortin (POMC). In the rat and monkey, most neurons in the arcuate nucleus that express POMC also express leptin receptor (9, 16), and leptin treatment in ob/ob mice induces POMC gene expression (39, 46, 54). Evidence suggests that the melanocortin system (presumably comprised of POMC neurons in the arcuate nucleus) mediates at least part of the inhibitory effect of leptin on feeding (15, 18, 53). In mice, blockade of melanocortin receptors in the brain or ablation of the melanocortin-4 (MC-4) receptor by gene targeting results in obesity (15, 26, 31, 36), and, in humans, mutations in the POMC and MC-4 genes result in phenotypes with profound obesity (33, 55, 58). Although the role of melanocortins in the regulation of reproduction has remained relatively unstudied and somewhat controversial, α-MSH has been shown to stimulate gonadotropin secretion in humans (35). Therefore, it is plausible that the melanocortin system provides a common pathway whereby leptin influences ingestive behaviors and reproductive function.

We used two complementary strategies to test the hypothesis that the effects of leptin on the neuroendocrine-reproductive axis and feeding are mediated by the melanocortin system. First, we studied the ability of the melanocortin receptor antagonist SHU9119 (24) to block leptin’s ability to inhibit food intake and stimulate the reproductive system of the ob/ob mouse. Second, we investigated the ability of the melanocortin agonist MTII (24) to simulate the effects of leptin on
feeding and reproductive function in ob/ob mice. We present evidence that the melanocortin system plays an important role in mediating leptin’s central effects on ingestive behaviors, but reject the hypothesis that the melanocortinergic pathway is involved in mediating leptin’s action on the neuroendocrine-reproductive axis.

MATERIALS AND METHODS

Animals

Male ob/ob C57BL/6j mice (Jackson Laboratories, Bar Harbor, ME) were housed individually in a 12:12-h light-dark cycle (lights off at 1800) with access to standard rodent chow and water ad libitum. The University of Washington’s Animal Care Committee approved all experimental procedures.

Experimental Design

Experiment 1. This experiment was designed to test the hypothesis that melanocortins mediate the effects of leptin on reproduction and feeding by attempting to 1) block the effects of exogenously administered leptin with the melanocortin receptor (MC-R) antagonist SHU9119 and 2) mimic the effects of leptin by giving the melanocortin agonist MTII to leptin-deficient ob/ob mice. Six-week-old ob/ob mice were divided into five weight-matched groups (n = 10/group). Mice were injected daily with a modified freehand intracerebroventricular technique 90 min before lights out with 5 µl of either artificial cerebrospinal fluid (aCSF), leptin (0.3 µg), SHU9119 (6 nmol), or MTII (6 nmol). Leptin, SHU9119, and MTII were dissolved in aCSF. The mice were injected for 10 days. The doses chosen were based on the results of previous studies and from our pilot studies where these doses were confirmed to have an effect on feeding behaviors in ob/ob mice.

Experiment 2. In this experiment, we sought to confirm the effects of MTII that had been observed in the first experiment, this time using a more rigorous treatment regimen. Five-month-old ob/ob mice were divided into two weight-matched groups and injected daily by freehand intracerebroventricular injection 60 min before lights out with 5 µl of either aCSF (n = 6) or 6 nmol MTII dissolved in aCSF (n = 7). Additionally, intraperitoneal injections of either 100 µl of saline (0.9%) or MTII (100 nmol) dissolved in saline (0.9%) were administered at 0700. The mice were injected for 10 days.

Experimental end points. Animals and food were weighed each evening at the time of injection. Additionally, on day 7 of experiment 1, food intake was recorded approximately every 90 min after injection for 9 h and then again at 14 and 24 h. Rectal temperatures were obtained from unanesthetized, hand-restrained animals every 2 days at 0700 for experiment 1. Daily water intake was recorded with the use of graduated water bottles for experiment 2.

Blood was taken on day 10 by orbital eye bleed at 2 h and 1 h before a third terminal bleed. The animals were killed by rapid decapitation. Serum was obtained with the use of Microtainer serum separators (Beckton Dickinson, Franklin Lakes, NJ) and frozen at −20°C until assayed for reproductive hormones. Retroperitoneal, inguinal, and epididymal fat pads were removed and weighed wet for experiment 1. Brains were removed and immediately frozen on dry ice. The brains were then sectioned (20 µm) with a cryostat through the lateral ventricle to confirm proper placement of the intracerebroventricular injection.

The testes, epididymides, and seminal vesicles were removed, fixed in Bouin’s solution, dissected, weighed, and embedded in paraffin before sectioning (2–4 µm) and staining with hematoxylin and p-aminosalicylic acid. Light microscopic examination of seminal vesicle and epididymis longitudinal sections and testis cross sections were performed to identify histological differences between groups. Additionally, the primary luminal area of the seminal vesicles was measured using the National Institutes of Health (NIH) Image computer program.

Freehand Intracerebroventricular Injections

The freehand intracerebroventricular injection technique was a modification of the Laursen and Belknap method (34). Mice were anesthetized with an isoflurane vaporizer (Veterinary Anesthesia Systems, Bend, OR) and placed in a sternal recumbent position. A 27-gauge ½-in.-long needle fitted with a 0.8 cm plastic sheath (leaving 0.4 cm needle exposed) was attached to the Luer-Lok hub of a 25-µl Hamilton syringe. Slight pressure was applied to the ears in a downward direction to level and stabilize the head during injection. The injections were made into the lateral ventricle with the needle inserted perpendicularly to the head, 1.0 mm posterior of bregma and 0.5 mm lateral to the midline. An initial hole was made 1 day before the beginning of the study, and all further injections were made through the same hole. After a slow continuous injection, the needle remained in place for several seconds, allowing the solution to disperse and preventing backflow up the needle track.

Hormone Assays

Serum was assayed for luteinizing hormone (LH) and follicle-stimulating hormone (FSH) by RIA with reagents from NIH. The standards used for FSH were rFSH-RP2 (antiserum anti-rFSH-S11). The standards used for LH were rLH-RP3 (anti-rLH-S11). Tracers for both assays were obtained from Corning-Hazelton (Vienna, VA). The intra-assay coefficients of variation (cv) for LH and FSH were 16 and 11%, respectively. Testosterone was assayed by using the Delphi fluorimunnoassay (EG&G Wallac, Turku, Finland). The interassay cv for the testosterone assays was 13.1%.

Statistical Analysis

For all experiments, n is the number of experimental animals within a group, and this was the n used in the analysis. Of the 60 animals in experiment 1, one died and one became sick during the study; these two animals were excluded from all statistical analyses. All data are expressed as means ± SE for each group. In experiment 1, the differences between groups were assessed by ANOVA. When the ANOVA indicated a significant difference, Fisher’s protected least-significant difference test was used to identify differences between individual groups. In experiment 2, Student’s unpaired t-test was used to determine significant differences between groups. The rejection level for all statistical tests was set at α = 0.05.

RESULTS

Experiment 1

Leptin treatment resulted in the expected reduction in food intake compared with vehicle (−70%; P < 0.0001) and also reduced body weight (P < 0.0001; Figs. 1 and 2). When SHU9119 was given together with leptin, this treatment increased food intake relative to leptin given alone (+38%; P < 0.05) and attenuated leptin’s weight loss effects (P < 0.0001). Given alone,
SHU9119 reduced food intake compared with vehicle (−17%; *P* < 0.01) but had no effect on body weight (*P* = 0.40). MTII treatment reduced food intake relative to vehicle (−15%; *P* < 0.01) and also reduced body weight (*P* < 0.001).

To examine the time course of the changes observed in food intake between treatment groups, we measured food consumption at several time points after injection (Fig. 3). At 1.5 h postinjection, all treatment groups ate less than the vehicle-treated group (*P* < 0.05); however, this was the only time point for which the SHU9119-treated group was significantly different from the vehicle-treated animals (data not shown). At 1.5, 3, and 5 h after injection, the MTII-treated group was not different from the leptin group, showing the short-term feeding-inhibitory effect of this compound. However, from 6.25 h on, the MTII-treated animals ate more than the leptin-treated group (*P* < 0.05), and, by 24 h, their cumulative food intake was similar to that of the vehicle group. For the first 9 h, food intakes of the leptin and leptin/SHU9119 groups were not significantly different, but from 14–24 h, the leptin/SHU9119-treated animals ate significantly more than the leptin group (*P* < 0.01), showing the sustained duration of the effect of SHU9119 on the feeding axis.

Rectal temperatures were obtained every 2 days to monitor changes during the course of treatment. By day 2, the vehicle-treated group had lower rectal temperatures than all other treatment groups (vehicle vs. leptin, leptin-SHU9119, SHU9119, *P* < 0.05; vehicle vs. MTII, *P* < 0.001), and this difference persisted at all time points measured (data not shown). Leptin treatment resulted in higher body temperatures on days 4, 6, and 8 compared with all other treatment groups, reflecting the thermogenic properties of this hormone. By the end of the experiment, however, the temperature difference between the leptin- and leptin-SHU9119-treated groups had disappeared and both groups had significantly higher temperatures than all other treatment groups. Overall, for the entire period of the study, the leptin and leptin-SHU9119 groups had positive temperature changes greater than any other treatment. Groups that do not share a common superscript are significantly different from one another at *P* < 0.05. Values are presented as means ± SE in °C (n = 9 or 10 animals/group).

Fig. 1. Experiment 1 mean daily food intake. Leptin-treated groups had significantly reduced food intake compared with all other groups. Groups that do not share a common superscript are significantly different from one another at *P* < 0.05. Values are presented as means ± SE in g (n = 9 or 10 animals/group).

Fig. 2. Experiment 1 total 10-day body weight change. Leptin-treated groups had a greater loss of body weight compared with all other groups. Groups that do not share a common superscript are significantly different from one another at *P* < 0.05. Values are presented as means ± SE in g (n = 9 or 10 animals/group).

Fig. 3. Experiment 1 time course of cumulative food intake for 24 h after injection. SHU9119 group was not significantly different from vehicle (except at 1.5 h after injection) and was omitted from graph for clarity. Leptin-treated groups had significantly reduced food intake compared with all other groups. Values are presented as means ± SE in g (n = 9 or 10 animals/group).

Fig. 4. Experiment 1 total 10-day change in rectal temperature from day 0. Leptin and leptin/SHU9119 treatments produced greater temperature gains than any other treatment. Groups that do not share a common superscript are significantly different from one another at *P* < 0.05. Values are presented as means ± SE in °C (n = 9 or 10 animals/group).
Combined fat pad weights were highest in the SHU9119-, MTII-, and vehicle-treated groups, which were not significantly different from each other. Fat pad weights for these three groups were all higher than either leptin-SHU9119 (P < 0.05)- or leptin-treated groups (P < 0.001; Table 1).

Gonadotropin levels were elevated only in the leptin- and leptin-SHU9119-treated groups. Serum FSH levels were highest in the leptin (+97% vs. vehicle-) and leptin-SHU9119 (+94% vs. vehicle)-treated groups, which were both significantly higher (P < 0.0001) than in any other treatment (Fig. 5). Serum LH levels were higher only in the leptin-treated group (Table 1), although the overall ANOVA did not reach significance (P = 0.12). Because of insufficient blood volumes, testosterone levels were not measured in this experiment.

Testicular weights were not significantly different among the groups (Table 1). Seminal vesicle weights, however, were significantly greater in the leptin- and leptin-SHU9119-treated groups than in the other three treatment groups (P < 0.001). Epididymal weights were moderately elevated in the SHU9119 group compared with all other treatment groups (P < 0.05). Histological analysis of the seminal vesicles revealed that primary lumen size was virtually identical in the two leptin-treated groups, and was significantly enlarged compared with the other treatment groups (+1,100% vs. vehicle, +2,900% vs. SHU9119, +1,300% vs. MTII; P < 0.0001; Fig. 6). No obvious differences were observed in testicular or epididymal morphology among the groups. All stages of spermatogenesis were present in the testes, and abundant mature sperm were evident in the epididymides of all groups.

**Experiment 2**

After 10 days of combined intraperitoneal and intracerebroventricular treatment, activation of MC receptors by MTII resulted in lower cumulative food intake (–27% vs. vehicle; P < 0.01; Fig. 7) and in lower body weights (P < 0.01; Fig. 8). Water intake was not significantly different between groups (Table 2).

As was seen in the first experiment, MTII treatment did not cause a change relative to vehicle treatment in either FSH levels (P = 0.80; Fig. 9) or in testosterone levels (P = 0.16; Table 2). Testicular and epididymal weights were not different between the MTII- and vehicle-treated groups, although the vehicle-treated group did have significantly greater seminal vesicle weights (Table 2). However, no differences were observed in the size of the seminal vesicle lumens between the MTII- and vehicle-treated groups (P = 0.26), concurring with results of the first experiment (Fig. 10).

Qualitative morphological observation of the testes revealed no obvious differences between the two groups, with all stages of spermatogenesis being present, no apparent degeneration of Leydig cells, and only minor Sertoli cell pyknosis seen in both groups. Additionally, some phagocytosing of spermatids was observed in the seminiferous tubules of both treatment groups.
The melanocortin receptor antagonist SHU9119 given alone had only a slight effect on either feeding or body weight in these mice, presumably reflecting the fact that the melanocortin pathway, a putative inhibitor of the feeding axis, is itself rendered quiescent by the lack of leptin in ob/ob mice (28). It is interesting to note that the SHU9119 treatment, although having no overall effect on body weight, did moderately reduce food intake compared with vehicle-treated animals. This may reflect the reported partial agonist activity of SHU9119 at the MC-3 receptor (24, 45), which could lead to a mild inhibition of feeding compared with the vehicle-treated controls.

The effects of leptin on food intake and body weight have been well documented (4, 42). ob/ob Mice treated with leptin either centrally or peripherally show dramatic decreases in feeding and adiposity, leading to sustained lower weights for as long as leptin is administered. We show here that chronic intracerebroventricular treatment with leptin had a similar effect, with no sign of the leptin resistance seen in other models of murine obesity (21). When we gave leptin and SHU9119 together, the MC-R antagonist significantly attenuated the chronic inhibitory effects of leptin on feeding and body weight. This extends the findings of previous reports documenting an acute blockade of the actions of leptin in rats that are pretreated with SHU9119 (44, 48). However, the observation that SHU9119 does not completely block the effects of leptin in feeding behavior indicates that the melanocortin system probably represents only one of several pathways through which leptin acts.

The time course of MTII and SHU9119 actions on the feeding axis revealed that, although the effects of SHU9119 were long lived, the actions of MTII were most apparent soon after treatment. When we measured the time course of MTII actions after injection, we found that, although food intake in the MTII group was inhibited for the first 6 h after injection, the MTII-treated mice ate more than vehicle-treated mice for each time period thereafter, leading to little net change in cumulative food consumption between groups over 24 h. In the only other study reported thus far where MTII was administered to ob/ob mice, MTII was seen to inhibit food intake for up to 12 h (15), but time points after this were not examined. In the same study, wild-type mice were found to normalize their food intake 8 h after MTII treatment, which agrees closely with the observations we report here. In contrast, studies in rats have shown that a single injection of MTII causes a dose-dependent inhibition of food intake consistent with the neuroendocrine-reproductive axis.

**DISCUSSION**

In this study, we demonstrated that high-affinity agonists and antagonists of melanocortin receptors alter feeding behavior and body temperature in the ob/ob mouse; however, these same agents act neither alone nor in combination with leptin to influence the status of the reproductive endocrine system of the ob/ob mouse. On the basis of these observations, we deduce that, although central circuits involving the melanocortin system appear to mediate the effect of leptin on feeding and metabolism, we could adduce no evidence that these same pathways link leptin’s action to the neuroendocrine-reproductive axis.

**Food Intake and Body Weight**

Our results confirm earlier short-term (24 h) studies in both the rat and mouse, which show that the melanocortin agonist MTII and the antagonist SHU9119 acutely inhibit and stimulate feeding, respectively (15, 18, 44, 48, 53). Our studies extend these observations by showing the long-term effects of these compounds on the ingestive axis. We have demonstrated that MTII inhibits food intake, both acutely and chronically, when it is given directly into the brain and that the chronic effects of centrally administered MTII on food intake and body weight are potentiated by an additional peripheral (intraperitoneal) injection of MTII 12 h after the intracerebroventricular dose. This “booster dose” was given intraperitoneally rather than intracerebroventricularly to limit the animals’ exposure to anesthesia and to reduce the risk of trauma associated with central injections. Before embarking on the experiments reported here, we performed a pilot study giving a peripheral dose of MTII once daily for 9 days. We found that with intraperitoneal injections alone, MTII inhibited food intake acutely; however, the animals rebounded after several hours and then actually consumed more than a normal amount of food during the subsequent light phase, resulting in no net effect of MTII on chronic food intake or body weight over the 24-h period (data not shown).

The melanocortin receptor antagonist SHU9119 given alone had only a slight effect on either feeding or body weight in these mice, presumably reflecting the fact that the melanocortin pathway, a putative inhibitor of the feeding axis, is itself rendered quiescent by the lack of leptin in ob/ob mice (28). It is interesting to note that the effects of MTII and SHU9119 on chronic food intake or body weight over the subsequent light phase, resulting in no net effect of SHU9119 at the MC-3 receptor (24, 45), which could lead to a mild inhibition of feeding compared with the vehicle-treated controls.
for up to 48 h (53). Although we did not examine the time course of a single injection in this study past 24 h, the compensatory overfeeding during the daytime exhibited by ob/ob mice would suggest that, in this model, the effects of MTII are shorter lived in the mouse than in the rat. The effects of SHU9119 in the rat are evidently longer lasting, with stimulation of food intake for up to 96 h after a single dose (18). In this study, we report for the first time the chronic effects of this compound in a model of leptin deficiency. Here the time course of SHU9119 actions observed in the ob/ob mouse would be consistent with experiments in the rat, where SHU9119 attenuates leptin’s satiety effects both acutely and chronically. Whether this long-lived effect of SHU9119 on the feeding axis reflects the continued antagonism of MC-R or the sustained actions of downstream targets is as yet unknown.

Body Temperature

We also found that leptin treatment increased rectal temperatures, in agreement with previous reports showing a normalization of body temperature in ob/ob mice after leptin injections (22). We were interested to observe a partial thermogenic effect of MTII and no apparent effect of the blockade of MC-Rs on temperature in this model. α-MSH has been shown to have antipyretic activity in many species (6) and is reported to have a hyperthermic effect in afebrile rats (43). A recent report by Huang et al. (25) shows that α-MSH, although preventing fever in endotoxin-treated rats, has no effect on temperatures of normal animals. In the same study, SHU9119 was shown to be fever promoting in endotoxin-treated rats, but had no effect in afebrile animals. Our demonstration of the thermogenic actions of MTII in the ob/ob mouse, albeit not as robust as leptin’s, lends further credence to the importance of melanocortins in regulating body temperature.

Both leptin and the melanocortin agonist MTII are clearly thermogenic; however, in our studies, treatment with the melanocortin antagonist SHU9119 did not alter body temperatures relative to vehicle-treated controls and SHU9119 did not attenuate the thermogenic action of leptin. This apparent contradiction between the thermogenic effect of a melanocortin agonist and no effect of a melanocortin antagonist on body temperature poses questions that deserve further exploration, perhaps with a first step being a careful dose-response study of both compounds. One plausible explanation for the lack of an effect by SHU9119 on temperature (as well as on body weight), aside from the question of dosage, might be that levels of agouti-related protein (AgRP), the postulated endogenous MC-R antagonist, are upregulated 10-fold in ob/ob mice (41, 50). The high levels of AgRP expression would cause a tonic inhibition of MC-R activation, and thus may be the reason that SHU9119 is by itself ineffective, given the inference that the MC-Rs are fully antagonized by AgRP. Arguing against this hypothesis, however, is a recent report that leptin treatment lowers AgRP gene expression dramatically in the ob/ob mouse (57). Whether it is the alteration in AgRP or some other mechanism that is responsible for the lack of an effect by SHU9119 on thermogenesis, it is clear that ob/ob mice modulate their body temperatures in markedly different ways than do normal mice. Thus it may be that other rodent models may respond differently. However, in our study, leptin’s effect on body temperature was not influenced by SHU9119 when it was administered at a dose that mitigated the effects of leptin on feeding and body weight.

Water Intake

We were surprised in experiment 2 that water intake did not differ between MTII- and vehicle-treated animals. In our pilot studies with leptin, we consistently saw a reduction in water consumption commensurate with a reduction in food intake (unpublished observations). Thus we expected to observe the same response

Table 2. Experiment 2 water intake, reproductive organ weights, and testosterone levels

<table>
<thead>
<tr>
<th>Groups</th>
<th>Water Intake, g</th>
<th>Testes, g</th>
<th>Epididymis, g</th>
<th>Seminal Vesicles, g</th>
<th>Testosterone, ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>71.7 ± 3.1</td>
<td>0.097 ± 0.002</td>
<td>0.023 ± 0.003</td>
<td>0.24 ± 0.02</td>
<td>0.061 ± 0.041</td>
</tr>
<tr>
<td>MTII</td>
<td>68.3 ± 2.8</td>
<td>0.083 ± 0.009</td>
<td>0.023 ± 0.002</td>
<td>0.18 ± 0.01*</td>
<td>0.194 ± 0.055</td>
</tr>
</tbody>
</table>

Data are presented as group means ± SE. *P < 0.05 vs. vehicle.
in MTII-treated animals, which ate significantly less than the vehicle-treated group. Notwithstanding, our findings are in accord with the only other published study to date examining the effects of MTII on the drinking response of mice (15), wherein water intake is only mildly inhibited during the first hour after injection, but not at any other subsequent time point measured up to 7 h posttreatment. In another study conducted in rats, MTII injections into the lateral ventricle increase water consumption acutely, but decrease water intake over 24 h in a dose-dependent fashion (16). Our results suggest that in the absence of leptin, MTII has little effect on fluid intake in the ob/ob mouse, although it is conceivable that conducting a full dose-response curve of MTII and fluid intake might sort out these discrepancies.

Melanocortins and Reproduction

Despite clear evidence for melanocortins having an effect on feeding and thermogenesis, we adduced no evidence for an interaction between either the activation or blockade of the melanocortin system and leptin signaling to the reproductive axis. The melanocortin agonist MTII did not alter circulating levels of either the gonadotropins or testosterone and had no effect on morphology of the seminal vesicles. The melanocortin antagonist SHU9119, given either by itself or with leptin, had no significant effect on any aspect of reproductive function. These results are consonant with the recent reports of mutations in the human POMC and MC-4 receptor genes (33, 55, 58). Homozygous mutations by transversion or deletion of the POMC gene lead to extreme obesity, adrenal insufficiency, and pigmentations defects, but do not apparently cause reductions in non-POMC derived pituitary hormone levels. A heterozygous frameshift mutation in the human MC-4 receptor gene also results in obesity but not reproductive incompetence, because affected individuals are fertile, despite having body mass indexes of up to 41 kg/m². Ectopic expression of the agouti gene leading to constitutive blockade of melanocortin receptors is associated with a reduction in fertility in older obese Ay mice (17); however, it is unclear whether this occurs as a direct result of inactivation of MC-Rs or as a secondary effect related to the adult-onset obesity syndrome exhibited by these animals. It is conceivable that the doses of MTII administered in our current studies were either insufficient to have had an effect on the reproductive axis or that the injection paradigm was not optimal to reveal such an action. Even so, it is notable that in several studies, even very high doses of the endogenous agonist α-MSH, given either peripherally or into the median eminence, have not revealed any inductive effects on reproductive parameters in rodents (30, 47, 49).

Leptin and Reproduction

Although our results suggest that leptin’s effects on reproduction are not mediated by melanocortinergic pathways, we have demonstrated here that central injections of leptin stimulate the reproductive axis in male ob/ob mice. This observation, which suggests a central action of leptin in the regulation of reproduction, is consistent with the earlier work of Swerdloff et al. (51), who found that the reproductive deficits in the ob/ob mouse could be attributable, in part, to hyposensitivity of the brain-pituitary axis to the negative feedback effects of sex steroids. Further evidence that leptin acts directly on the brain to regulate reproduction comes from studies showing that leptin acutely stimulates LH secretion in ovariectomized rats (59) and that intracerebroventricular injections of leptin can induce sexual maturation in food-restricted peripuberal rats (19). The latter study established that chronic injections of high doses (10 µg/day) of leptin into the lateral ventricle allow food-restricted prepuberal rats to progress through puberty, whereas their saline-treated, food-restricted counterparts fail to sexually mature. It has also been demonstrated that central injections of leptin antisera can inhibit LH pulsatility in rats (5, 32). Here we show that relatively small doses (0.3 µg) of leptin, administered centrally, can also stimulate the hypothalamic-pituitary-gonadal axis in mice. There are leptin receptors in the pituitary (3), and we cannot exclude the possibility that the effects we observed are attributable, at least in part, to the diffusion of leptin into the hypophysial portal circulation producing a direct action of leptin on pituitary gonadotropes (59).

The possibility also remains that some of the effects of leptin on the reproductive system may have been induced by leakage of leptin back into the general circulation, where it could act directly on the testes and accessory reproductive tract, which have been shown to express leptin receptors (10, 23). This seems unlikely, however, because Gruaz et al. (19) found no increase in plasma levels of leptin after an intracerebroventricular dose of 10 µg leptin, a 30-fold higher dose than we used in this study. Nevertheless, another recent study found some radiolabeled leptin administered intracerebroventricularly into mice does appear in the general circulation, probably though reabsorption of cerebrospinal fluid (37), suggesting that it is at least conceivable that some of the leptin delivered into the ventricles in our study did appear in the general circulation. Although leakage of leptin into the circulation could have contributed to the activation of gonadal function seen in this experiment, in our hands, a single intraperitoneal injection of 50 µg leptin daily for 10 days was insufficient to restore any reproductive parameter in ob/ob mice (unpublished observation). Therefore, it seems unlikely that leakage from the brain of 0.3 µg or less would have had any significant effect.

In agreement with our previous study (2), leptin treatment had a greater effect on FSH levels than on LH levels in male ob/ob mice. This may be due to the relatively poor resolution of the (too infrequent) sampling technique, which makes it difficult to detect small increases in mean plasma LH levels in the context of its pulsatile release mode (12, 59). The apparent lack of effect of leptin on serum LH levels may be attributable
either to this resolution problem or conceivably to the confounding effects of anesthesia and stress. These factors could also have influenced the FSH results, but perhaps the more “tonic” nature of FSH secretion and its more stable baseline were easier to resolve with the limited, pooled blood-sampling technique used here. The approximate doubling of serum levels of FSH found in the leptin-treated group compared with the vehicle-treated group indicates a strong inductive effect of leptin on FSH release. We cannot be certain whether the observed increased blood FSH concentrations translate into facilitation of testosterone production, due to the lack of blood available for testosterone measurements in experiment 1. The histological examination of testes and epididymides leads us to conclude that the reproductive deficit in very young male ob/ob mice is not due to gross defects at this level, as no obvious differences were observed in testicular morphology, spermatogenesis, or in sperm storage in the epididymis. This suggests that male ob/ob animals have sufficient levels of circulating testosterone to permit at least some functional gonadal activity and that perhaps their reproductive deficit lies elsewhere. However, the seminal vesicles of the young ob/ob animals in our studies do respond robustly to leptin treatment, as evidenced by the increases in both seminal vesicle weight and luminal size, which can be reasonably deduced to reflect the inductive action of testosterone (13).

In summary, we have shown that chronic central injections of leptin stimulate the reproductive axis of male ob/ob mice at a dose that has no effect when administered peripherally. We have also shown that, whereas melanocortins and their receptors are clearly involved in mediating the action of leptin on feeding and body weight, the leptin-dependent activation of the reproductive system is unlikely to be transduced through these same melanocortin pathways. This apparent dissociation of central pathways through which leptin exerts its actions on feeding and reproduction lends additional insight into the specific hypothalamic circuits that link leptin to the regulation of the diverse and complicated neuroendocrine control systems resident in the hypothalamus.

Perspectives

Our results suggest that the melanocortin signaling pathway, at least in the male ob/ob mouse, does not mediate leptin’s tonic activation of the reproductive axis. However, this would not rule out a possible role for melanocortins in regulating other brain-dependent reproductive events. For example, a recent report by Watanobe et al. (56) shows that blockade of MC-3 and/or MC-4 receptors reduces the amplitude of the steroid-induced LH surge in ovariectomized rats. Although the neuroendocrine mechanisms for generating the LH surge in the female are different from those regulating testicular function in the male, the results of Watanobe et al. underscore the potential complexity involved in unraveling the functional significance of the melanocortins in the regulation of neuroendocrine function. Further studies will be required to elucidate the molecular mechanisms through which the melanocortinergic system exerts its effects in the brain in both males and females. Many neuropeptides have been shown to exhibit differing effects on LH secretion due to hormonal status or variations in route of administration, dosage, or duration, and this could conceivably also be the case for α-MSH. Whereas, our own study revealed no apparent effect of either activation or blockade of melanocortin receptors on rescuing reproductive function in the male ob/ob mouse, it remains plausible that, under certain circumstances, melanocortins serve an important role in mediating leptin’s action on specific neuroendocrine events, such as the proestrus LH surge. In addition, it is conceivable that other POMC cleavage products, notably β-endorphin, may be involved in regulating the stimulatory effects of leptin on the reproductive axis, either positively or negatively. Also, because the majority of hypothalamic POMC neurons expresses the recently characterized neuropeptide cocaine and amphetamine regulated transcript, a careful study of the neuroendocrine actions of this peptide is certainly warranted.

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