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Opioid Receptor Blockade Promotes Weight Loss and Improves the Display of Sexual Behaviors in Obese Zucker Female Rats

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MARÍN-BIVENS, C. L. AND D. H. OLSTER. *Opioid receptor blockade promotes weight loss and improves the display of sexual behaviors in obese Zucker female rats.* PHARMACOL BIOCHEM BEHAV **63**(3) 515–520, 1999.—Obese Zucker female rats are hyperphagic, overweight, infertile, and hyporesponsive to the inductive effects of ovarian steroid hormones on sexual behaviors. It has been postulated that endogenous opioid activity may contribute to their obesity and reproductive dysfunction. To test this hypothesis, ovariectomized, adult obese Zucker rats were treated with the opioid receptor antagonist, naltrexone, or saline prior to measurement of steroid-induced sexual behaviors, food intake, and body weight. In estradiol benzoate (EB)-treated rats, naltrexone injection increased the display of sexual receptivity (lordosis quotient, LQ: saline, 11 ± 10%; 5 mg/kg naltrexone, 54 ± 15%, $p < 0.05$) and also elicited proceptivity (PRO), which was never observed after saline injection. In EB plus progesterone-treated animals, naltrexone administration enhanced both sexual receptivity and proceptivity (LQ: saline, 17 ± 10%; 5 mg/kg naltrexone, 96 ± 3%; $p < 0.05$; PRO: saline, 3.0 ± 2.4 bouts/min; 5 mg/kg naltrexone, 45.3 ± 12 bouts/min; $p < 0.01$). Naltrexone injection also decreased 24-h food intake (saline, 24.2 ± 0.7 g; 5 mg/kg naltrexone, 17.6 ± 1.2 g; $p < 0.05$) and weight change (saline, + 7.3 ± 0.8 g; 5 mg/kg naltrexone, −4.5 ± 1.4 g, $p < 0.01$). Morphine treatment blocked these effects of naltrexone on sexual behaviors, food intake, and body weight. These data suggest that endogenous opioids contribute to hyperphagia, obesity, and behavioral hyporesponsiveness to ovarian steroid hormones in obese Zucker rats. © 1999 Elsevier Science Inc.

Body weight Food intake Naltrexone Obesity Opioid Proceptivity Receptivity Sexual behaviors Zucker rats

OBESITY in Zucker rats is inherited as a Mendelian, homozygous recessive trait (*fa/fa*), which is coupled with several physiological and behavioral abnormalities, including elevated serum corticosterone, insulin, and lipid levels, enlarged adipose tissue stores, lowered whole-body metabolism and thermoregulation, and excessive feeding (2,49). Obese Zucker female rats are sterile, showing numerous reproductive abnormalities including delayed vaginal opening, abnormal estrous cyclicity, inadequate reproductive behaviors when introduced to sexually active male rats, and failure to conceive (4,38,49). When treated with estradiol and progesterone, ovariectomized (OVX) obese Zucker rats display significantly less reproductive behavior than lean Zucker females (27).

Abnormal reproductive function and energy regulation in obese Zucker rats may be triggered by hyperactive neurotransmitter systems, such as the endogenous opioids, which are inhibitory to reproduction and stimulatory to weight gain. Obese Zucker rats have elevated concentrations of β-endorphin in whole brain and pituitary, and higher hypothalamic and/or midbrain levels of Met-enkephalin and dynorphin, compared to lean Zucker rats (12,25,29,37). Administration of opioid agonists increases food intake and/or weight gain in lean rats (14,31), while treatment with opioid receptor antagonists reduces feeding and weight gain in lean and obese Zucker rats (7,25,28). Numerous investigators have reported that endogenous and exogenous opioids also influence reproductive behaviors in lean rats. In general, injection of opioid agonists, particularly those specific to the μ receptor subtype, results in a suppression of steroid-induced sexual behaviors; conversely, treatment with opioid receptor blockers enhances the display of sexual behaviors in OVX rats receiving subthreshold doses of ovarian steroid hormones (33,47). However, it is not yet known whether opioid receptor antagonism affects sexual behavior in obese Zucker female rats. This

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study was designed to confirm prior observations that opioid receptor blockade decreases food intake and body weight in obese Zucker rats, and to test the hypothesis that this treatment would improve the display of steroid-induced sexual behaviors in females of this genotype.

METHOD

Animals, Housing, and Surgery

Nine-week-old obese Zucker female rats (n = 20) were purchased from the Department of Nutrition, University of California, Davis. Sexually experienced Long–Evans rats (4–6 months old, 500–670 g from Charles River Laboratories, Wilmington, MA) were used as stimulus males for the behavioral tests. All animals were housed singly in a room with ambient temperature at 22–23°C and a 14:10-h light:dark photoperiod (lights on 2300–1300 h). They were given unlimited access to food (Rodent Chow #5001, PMI Feeds, St. Louis, MO) and water. All procedures were approved by the University of California, Santa Barbara Animal Care Council, and followed the guidelines established by the NIH Guide for the Care and Use of Laboratory Animals. At 10.5 weeks of age, the female rats were anesthetized with 90 mg/kg ketamine (IP) and 2 mg/kg xylazine (IP), and bilaterally OVX with caution to prevent excessive adipose tissue removal.

Behavioral Observations

Two weeks after OVX, the rats were tested for the display of estradiol-induced and progesterone-facilitated sexual behaviors after saline, naltrexone, or naltrexone plus morphine treatment. All drugs and hormones were purchased from Sigma Chemical Co. (St. Louis, MO). One group of rats (n = 10) received estradiol benzoate (EB, 15 μg/kg, SC, dissolved in sesame oil) 24 h before behavioral observations. The other group (n = 10) received 15 μg/kg EB 24 h and progesterone (P, 2 mg/kg, SC, dissolved in propylene glycol) 4 h before behavioral observations. These hormone doses produce physiological levels of serum steroid hormones and induce maximal sexual behaviors in OVX lean rats, but generate supraphysiological levels of estradiol and physiological levels of P, yet stimulate sexual behaviors poorly, in OVX obese females (27).

Naltrexone solutions (naltrexone hydrochloride, dissolved in 0.9% saline, pH 7.0–7.4) were freshly prepared immediately before each experiment. Two hours before behavioral testing rats were injected with 0.9% saline or 1, 3, or 5 mg/kg naltrexone. All injections were given as 1 ml/kg SC. Morphine (15 mg/kg) (morphine hydrosulfate, 15 mg/ml in 0.9% saline, pH 7.0–7.4) or 0.9% saline (1 ml/kg) was injected SC 75 min after the initial naltrexone or saline administration. This protocol yielded five drug treatment groups: saline + saline, 1 mg/kg naltrexone + saline, 3 mg/kg naltrexone + saline, 5 mg/kg naltrexone + saline, and 5 mg/kg naltrexone + 15 mg/kg morphine. The five treatments were administered to each rat in a counterbalanced order to eliminate carryover effects of drug treatment, and were separated by a 1-week interval to allow for complete metabolism of steroid hormones, naltrexone, and morphine.

Reproductive behaviors in response to stimulus male rats were observed in Plexiglas cylinders (46 × 92 cm, American Plastics Corp., Camarillo, CA) with pine shavings on the floor. A dim red light (25 W) provided illumination during testing (1400–2200 h). Behavioral observations were made with the observer blind to the animals' experimental condition. Sexual receptivity was quantified as lordosis quotient (LQ, positive lordosis responses/10 mounts × 100%) and lordosis rating [LR, degree of arching of the back, 0 = no, 1 = slight, 2 = moderate, 3 = strong lordosis, respectively; (15)]. Proceptivity rate (pro) was measured as the frequency of ear-wiggling and hop-darting bouts per minute (9).

One week after the sexual behavior tests were completed, 24-h food intake (corrected for spillage) and body weight were monitored, following the same drug treatment regimen (without hormone injections) described above. The use of OVX rats avoids any possible confound due to ovarian hormone modulation of food intake.

Data Analysis

One-way or two-way, repeated-measures ANOVAs and within-subjects LSD post hoc comparisons were used, as appropriate, to analyze body weight, food intake, and proceptivity data for effects of drug and hormone treatment. Friedman two-way ANOVA by ranks and multiple comparisons were used to evaluate sexual receptivity data for effects of drug treatment (40). For all analyses the criterion for statistical significance was $p < 0.05$.

RESULTS

In EB-treated, OVX obese Zucker rats, naltrexone administration increased sexual receptivity, compared to saline treatment (Fig. 1, LQ, $F_r = 148.4$, $p < 0.025$; LR, $F_r = 147.5$, $p < 0.01$), but only after injection of the 5 mg/kg dose (LQ and LR multiple comparisons, both $p < 0.05$). Remarkably, naltrexone treatment also induced proceptive behaviors, which were never observed in saline-treated obese rats receiving EB only (Fig. 1, ANOVA $F = 5.33$, $p < 0.0025$). Again, this was only true for the highest dose of the compound (5 mg/kg, within subject LSD $p < 0.05$). In OVX rats receiving EB plus P, the 5-mg/kg naltrexone treatment increased sexual receptivity, compared to saline administration (Fig. 2, LQ and LR multiple comparisons, $p < 0.05$) and all doses of naltrexone significantly increased ear wiggling and hop darting compared to saline treatment [Fig. 2, ANOVA $F(4, 76) = 5.33$, $p < 0.025$]. Proceptive behaviors following these three doses of naltrexone were not significantly different from each other (within subjects LSDs, all $p > 0.05$). All of the effects of naltrexone on the display of sexual behaviors were reversed by administration of morphine in both EB- and EB plus P-treated Zucker rats (Figs. 1 and 2).

All doses of naltrexone resulted in a reduction in 24-h food intake, compared to saline injection [Fig. 3, ANOVA, $F(4, 76) = 51.8$, $p < 0.0001$]; the 5-mg/kg dose of the drug was more effective than either the 1- or 3-mg/kg dose (within subject LSDs, both $p < 0.01$). Correspondingly, at all doses, naltrexone treatment curbed daily weight gain in obese Zucker rats [Fig. 3, ANOVA, $F(4, 76) = 24.43$, $p < 0.0001$]. In fact, the highest dose of naltrexone (5 mg/kg) actually promoted weight loss in these animals, and this suppressive effect on daily weight gain was greater than those observed following injection of the 1- or 3-mg/kg doses of the compound (within subject LSDs, both $p < 0.01$). All effects of naltrexone on food intake and weight gain were completely reversed by morphine administration (Fig. 3).

DISCUSSION

Steroid-Induced Reproductive Behaviors

In EB-treated OVX obese Zucker rats, systemic naltrexone administration enhanced sexual receptivity and also elicited the display of proceptivity. In females receiving EB plus

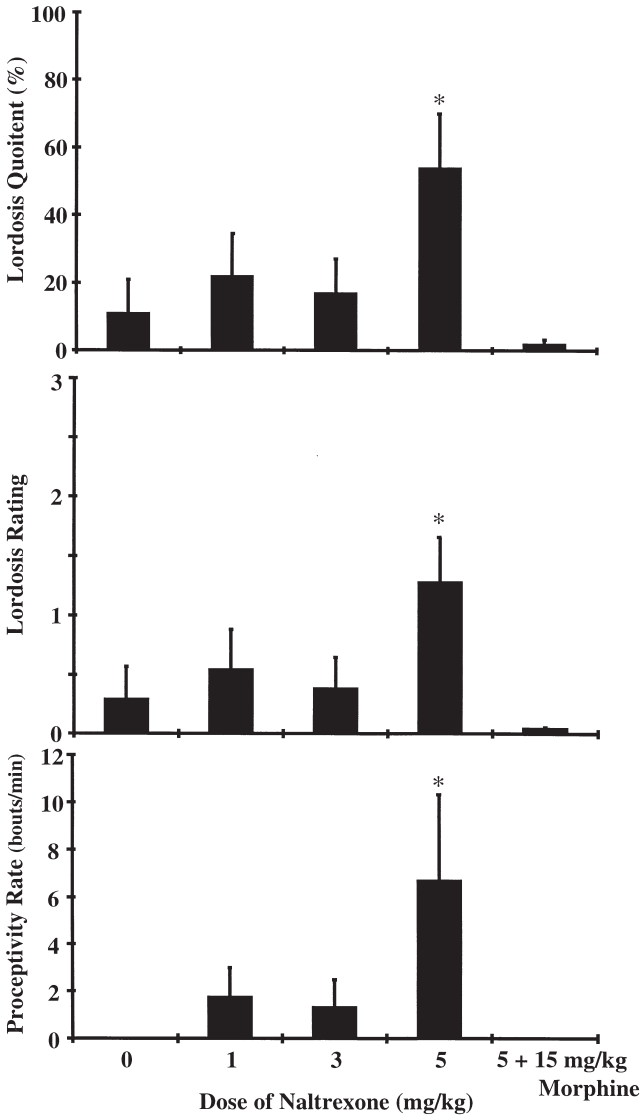

FIG. 1. Lordosis quotient (top), lordosis rating (middle), and proceptivity rate (bottom) in estradiol benzoate (15 μg/kg)-treated, ovariectomized, obese Zucker rats after systemic injection of saline, naltrexone, or naltrexone plus morphine. All data are mean ± SEM, $^*p < 0.05$ vs. saline treatment, $n = 10$ rats.

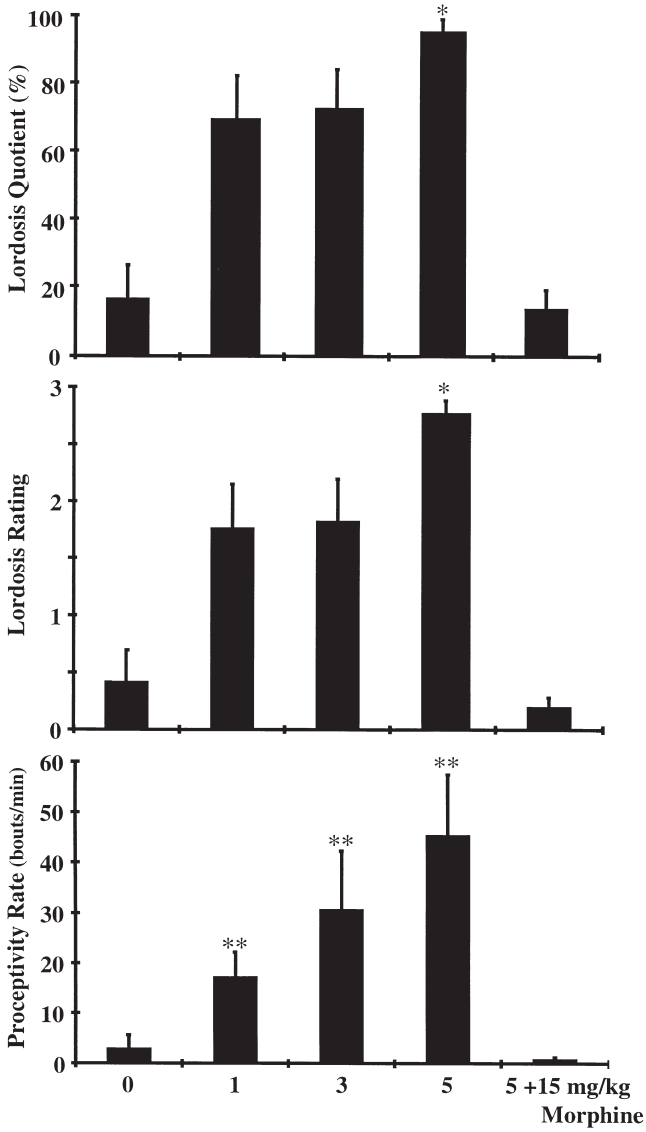

FIG. 2. Lordosis quotient (top), lordosis rating (middle), and proceptivity rate (bottom) in estradiol benzoate (15 μg/kg)- plus progesterone (2 mg/kg)-treated, ovariectomized, obese Zucker rats after systemic injection of saline, naltrexone, or naltrexone plus morphine. All data are mean ± SEM, $^*p < 0.05$, $^{**}p < 0.01$ vs. saline treatment, $n = 10$ rats.

P, naltrexone treatment enhanced the display of sexual receptivity and proceptivity. These effects of naltrexone were blocked by administration of morphine, the classic opioid agonist. These data support the hypothesis that endogenous opioids exert tonic, inhibitory control over the display of sexual behaviors in obese female Zucker rats. We did not include lean Zucker females in this study, but previously published work from this laboratory has shown that lean females treated with these doses of estradiol and progesterone are maximally receptive and proceptive (27). The enhancement of steroid-induced sexual behaviors in OVX lean females following opioid receptor blockade is only observed if the females are minimally receptive at the outset, due to treatment with low doses of steroid hormones (41,42). Because a rigorous assessment of sexual behaviors following a range of opioid antagonist doses and steroid hormone doses in lean and obese rats has not been reported, we cannot conclude definitively that *overactivity* of endogenous opioid systems tonically inhibits the display of steroid-induced sexual behaviors in obese Zucker rats.

The design of this study precludes determination of which endogenous opioid inhibits the display of sexual behaviors in these animals, but data from other laboratories suggest that β-endorphin, acting via μ receptors, may be responsible. Central administration of β-endorphin, or more specific μ-receptor agonists, decreases the display of steroid-induced sexual behaviors in OVX lean rats, and these effects are blocked by injection of μ-receptor specific antagonists (33,34,41,42,47). In contrast, injection of opioid agonists specific to other receptor subtypes either increases or has no effect on the dis-

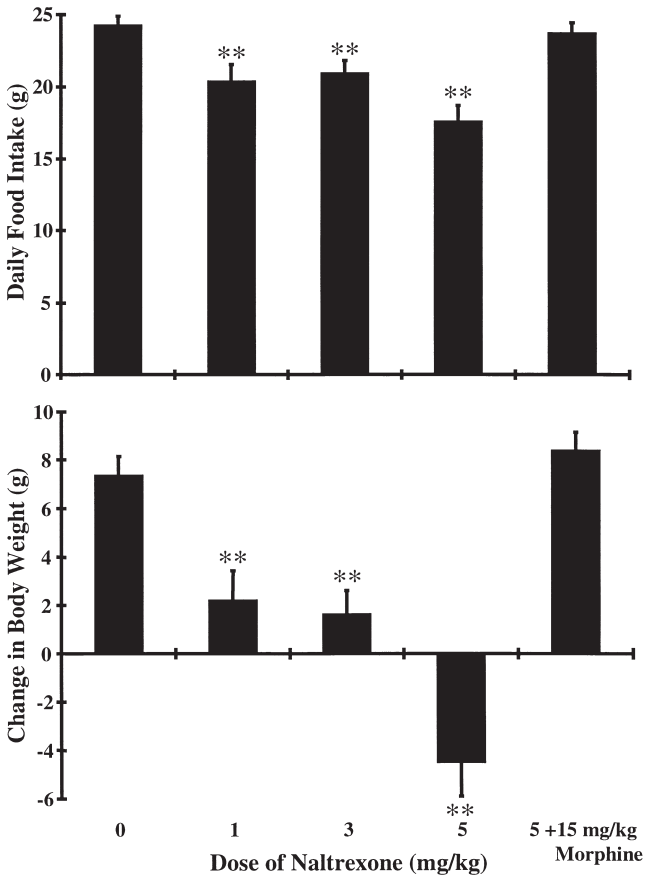

FIG. 3. Twenty-four-hour food intake (mean ± SEM, top) and change in body weight (mean ± SEM, bottom) in genetically obese Zucker rats after systemic injection of saline, naltrexone, or naltrexone plus morphine ($n = 20$). For each dependent variable, data points labeled with different letters differ significantly ($p < 0.01$).

play of steroid-facilitated sexual behaviors in lean females (33,34).

The site at which opioids exert these inhibitory actions in obese rats is not known. Injections of morphine into the ventromedial hypothalamus (VMH), or β-endorphin into the medial preoptic area (MPOA) or mesencephalic central gray (MCG), suppress the display of steroid-induced receptivity in lean female rats; these effects are reversed by administration of opioid receptor antagonists (41,42,45). β-Endorphin fibers and receptors are found in the MPOA and MCG, but not in the VMH of rats (20,24).

The suppression of sexual behavior that is observed following intracranial administration of opioid receptor agonists is reversible by injection of gonadotropin releasing hormone (GnRH); conversely, the facilitation of steroid-induced sexual receptivity noted following injection of the opioid antagonist, naloxone, is blocked by injections of GnRH antisera or GnRH antagonists (41–43). Ovarian steroids alter proopiomelanocortin gene expression in arcuate neurons projecting to the MPOA (6), and GnRH neurons in the POA receive synaptic input from β-endorphinergic fibers (5). This behavioral and neuroanatomical evidence supports the possibility of interactions among ovarian steroid hormones, GnRH, and β-endorphin neurons. Alternatively or additionally, opioids may suppress sexual receptivity in obese Zucker rats by modulating noepinephrine (NE) transmission. In lean female rats, the display of progesterone-facilitated lordosis is accompanied by increased NE release in the VMH (44). Systemic morphine injections inhibit both NE release in the VMH and the display of steroid-induced sexual behaviors (45).

Food Intake and Body Weight

Naltrexone treatment also curtailed daily food intake and weight gain in obese Zucker female rats in this study, effects that were reversed by morphine treatment. The naltrexone-induced changes in food intake that occur are presumably mediated by central opioid receptors, because methyl naltrexone, which does not cross the blood–brain barrier (19), does not affect food intake or body weight [for review see (31)]. Moreover, these effects of naltrexone on feeding and body weight are probably not caused by nausea because the doses of the compound used in this experiment do not produce conditioned-taste aversions (32). These data confirm previous reports that treatment with other opioid receptor antagonists decreases food intake in obese Zucker rats (7,25,28). Our data provide additional support for the general hypothesis that endogenous opioids contribute to the hyperphagia and obesity that characterize the obese Zucker genotype. Another animal model of obesity, the *ob/ob* mouse, has abnormally elevated plasma and brain concentrations of endogenous opioids, and treatment with naltrexone or other opioid antagonists also alleviates some aspects of their obesity (25,36). Plasma β-endorphin levels are higher in oligo-amenorrheic, obese vs. normal-weight women (13), but attempts to alleviate human obesity by administration of opioid antagonists have yielded mixed results (22). Thus, abnormally regulated endogenous opioids may be a common component of the manifestation of obesity, but amenability of the problem to pharmacological intervention with opioid receptor antagonists may differ, depending on species and/or etiology of the obesity.

The use of naltrexone and morphine in the current study precludes determination of which endogenous opioid(s) and opioid receptor subtypes are involved in the putative chronic, stimulation of food intake and weight gain in obese Zucker rats. Intracranial injection of μ, δ, or κ receptor-specific opioid agonists increases food intake in lean rats, and central injection of general or μ, δ, or κ receptor-specific opioid antagonists decreases feeding in lean and obese rats (7,14,31). Cannula mapping studies have shown that injection of opioid agonists to the nucleus accumbens, VMH, MPOA, paraventricular nucleus of the hypothalamus, ventral tegmentum, or hindbrain elicits food intake [reviewed in (14)]. These areas overlap with the distributions of β-endorphin, dynorphin, and enkephalin fibers and terminals, and the three opioid receptor subtypes [μ, δ, κ; (10,20,24,30)]. These data suggest that several endogenous opioids, acting through their respective receptor subtypes and multiple neural circuits, may contribute to hyperphagia and obesity in obese Zucker rats.

Opioid, Neuropeptide Y, and Leptin Interactions

The obese Zucker phenotype is due to a mutation in the gene encoding the leptin receptor, which results in a single amino acid substitution in the receptor protein, diminishing its ligand-binding ability and depressing its function (35). This mutated leptin receptor may preclude the normal, inhibitory action of leptin on neuropeptide Y (NPY) production in the hypothalamus (46). The resultant overproduction of NPY may be responsible for the hyperphagia, obesity, and reproductive dysfunction in obese Zucker females (1). Injections of

a putative NPY antagonist lowers food intake in obese Zucker males (17), and we recently reported that intraventricular injections of NPY antisera reduce food intake, promote weight loss, and improve the display of steroid-induced sexual behaviors in obese Zucker female rats (26). In lean rats, chronic, intraventricular injection of NPY stimulates feeding and weight gain, yet disrupts female reproductive function (3,48). Opioid receptor blockade attenuates the inductive effects of NPY on feeding and weight gain (21,23,39), suggesting that endogenous opioids mediate these actions of NPY. Centrally injected NPY stimulates β-endorphin release in the mediobasal hypothalamus of male rats (18). The demonstration of reciprocal connections between NPY and β-endorphin neurons in the arcuate nucleus (16) provides neuroanatomical evidence for the possible mediation by β-endorphin of NPY action on hypothalamically regulated behaviors. Interestingly, intraventricular injection of doses of leptin sufficient to reduce food intake does not improve the display of steroid-induced sexual behaviors in OVX obese Zucker rats (11), despite the fact that this protocol has been shown to lower food intake and hypothalamic NPY levels in male rats (8).

Our proposed model to integrate these observations posits that the leptin receptor mutation in obese Zucker rats may give rise to overproduction and excessive release of NPY in the hypothalamus, which in turn, may lead to overproduction of endogenous opioid peptides, including β-endorphin. Excessive opioid activity would then trigger hyperphagia, obesity, and reproductive aberrations in these animals. Our demonstration in the current study that opioid receptor blockade by naltrexone reduces food intake and body weight gain and stimulates the display of steroid-induced lordosis in OVX Zucker females is consistent with this hypothesis. Future studies will be required to establish the definitive links among endogenous opioids, NPY, and leptin, and to ascertain how these peptides may interact to trigger obesity and reproductive dysfunction in obese Zucker female rats.

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