Interactive effects of central leptin and peripheral fuel oxidation on estrous cyclicity

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Schneider, Jill E., and Dan Zhou. Interactive effects of central leptin and peripheral fuel oxidation on estrous cyclicity. Am. J. Physiol. 277 (Regulatory Integrative Comp. Physiol. 46): R1020–R1024, 1999.—A 48-h period of fasting inhibits estrous cycles in Syrian hamsters, and fasting-induced anestrus can be prevented by intracerebroventricular treatment with leptin during the fasting period. In the present experiment, the effects of intracerebroventricular leptin were blocked by systemic treatment with inhibitors of metabolic fuel oxidation. Leptin was infused continuously into the lateral ventricles (1 µg/day) during fasting on days 1 and 2 of the estrous cycle. Intraperitoneal injection of 2-deoxy-D-glucose (2DG) was used to block both central and peripheral glucose oxidation, and intragastric treatment with methyl palmitate (MP) was used to inhibit peripheral long-chain fatty acid oxidation during the fasting and leptin-treatment period. 2DG or MP were administered at doses that did not induce anestrus in ad libitum-fed hamsters. Despite elevated central levels of leptin, fasting-induced anestrus occurred in hamsters treated with either 2DG or MP. Thus an elevated intracerebroventricular leptin concentration is not a sufficient condition for normal estrous cycles when fuel oxidation is inhibited. These results raise the possibility that central leptin influences reproduction by indirect effects on peripheral fuel metabolism.

2-deoxy-D-glucose; methyl palmitate; ob protein; ovulation; sex behavior

LEPTIN, THE PROTEIN ENCODED by the ob (obese) gene, has received a great deal of attention for its putative role in control of energy expenditure, food intake, body weight (6, 7, 20, 25, 43), and reproductive function (1, 3, 10, 11, 15, 44). Research on leptin and related proteins may lead to new pharmacological treatments for obesity or infertility. It is therefore important to examine the effects of exogenous leptin treatment from a variety of different perspectives. Effects of leptin on aspects of reproductive function have been well documented in a variety of species (1–3, 8, 10, 11, 15, 29, 34, 35, 40, 44). For example, systemic treatment with murine leptin at 5 mg/kg reversed the effects of a 60-h fast on all aspects of estrous cyclicity in Syrian hamsters. Leptin treatment restored ovulation rate, sex behavior, precopulatory vaginal marking, and postovulatory vaginal discharge and decreased aggressive behavior (29). Before the discovery of leptin, it was demonstrated that changes in metabolic fuel availability and oxidation had profound influences on reproductive processes including luteinizing hormone (LH) and gonadotropin releasing hormone secretion (5, 17, 23, 27, 28, 32, 34, 41, 42, and J. E. Schneider, R. M. Blum, and G. N. Wade, unpublished observations). For example, estrous cycles and sex behavior in Syrian hamsters were interrupted by treatment with 2-deoxy-D-glucose (2DG) and methyl palmitate (MP), inhibitors of glucose and free fatty acid (FFA) oxidation, respectively (23, 27, 28, 30, 32–34). Given the effects of fuel metabolism on reproduction, it is interesting that leptin treatment has significant effects on metabolic fuel availability and oxidation (14, 16, 21, 22, 36, 38). Leptin can affect both reproductive and metabolic processes when applied directly to the central nervous system. For example, intracerebroventricular treatment with leptin can reverse the effects of fasting on sexual maturation in rats (19) and anestrus in adult Syrian hamsters (35). Acute intracerebroventricular treatment with leptin has long-lasting effects on food intake and body weight, energy expenditure, and lipid mobilization in peripheral tissues (21, 38). Microinjection of leptin into the ventromedial, but not the lateral hypothalamus, increases glucose uptake in a variety of peripheral tissues (24). Thus the central actions of leptin can affect food intake, energy balance, and reproduction via effects on peripheral metabolism. If so, it would be predicted that the effects of intracerebroventricular leptin treatment on estrous cyclicity would be prevented by treatment with metabolic inhibitors such as 2DG or MP. Alternatively, 2DG and MP treatment might fail to prevent the effects of leptin on reproduction. In the latter case, it would be concluded that a high level of leptin in the central nervous system is a sufficient condition for normal estrous cyclicity, even when fuel oxidation is inhibited.

MATERIALS AND METHODS

Animal care and use was in accordance with guidelines of the United States Department of Agriculture, National Institutes of Health, and the Lehigh University Institutional Animal Care and Use Committee. Female Lak:LVG Syrian hamsters, 75–95 g in weight, were purchased from either Harlan Sprague Dawley, Haslet, MI, or Charles River Breeding Laboratories, Wilmington, MA, housed individually in polypropylene cages in a room maintained on a 14:10-h light-dark cycle (lights out at 2300) at an ambient temperature of 22 ± 2°C, and fed Purina Laboratory Rodent Chow pellets (#5001) ad libitum unless noted otherwise. Syrian hamsters were used because they have a regular, 4-day estrous cycle that is essentially invariant under ad libitum feeding, but can be delayed by a number of factors that decrease energy availability (reviewed in 34, 41, 42). Sex behavior and ovulation normally occur during the evening of day 4. A copious vaginal discharge occurs on day 1 of the cycle, the day after ovulation and estrous behavior. Previous work showed that a 48- to 60-h period of fasting on days 1 and 2 of the cycle inhibited sex behavior, ovulation, and vaginal
discharge in lean, but not fattened, Syrian hamsters (32). These effects can be reversed by either intraperitoneal treatment at 10 mg/kg per day (30) or intracerebroventricular treatment at 1 µg/day with murine leptin during the 48- to 60-h period of fasting (35). Before the start of treatments, the expected time of ovulation and behavioral estrus was determined by examining the vaginal discharge each morning. Hamsters were used only if they showed two consecutive 4-day estrous cycles, including one positive test for sex behavior. Only hamsters that showed two consecutive 4-day cycles were used in this experiment.

Murine leptin (PeptoTech, Rocky Hill, NJ) was dissolved in artificial cerebrospinal fluid at pH 7.4. The leptin solution was delivered continuously at 1 µg per day via a minipump (Alza, model 1003D) designed to release 1 µl/h for 3 days. The minipumps were attached to 28-gauge cannulas (Alza) aimed at the left lateral ventricle. Vehicle-treated hamsters received artificial cerebrospinal fluid by the same route. Minipumps were implanted between the hours of 0900 and 1400 on day 4 of the estrous cycle to avoid the effects of anesthesia on the LH surge. Diffusion of leptin from the Alzet minipump begins 4 h after the pump is implanted. Thus infusion of leptin or vehicle from the pumps began between 1300 and 1800 on day 4 and continued on days 1 and 2, until between 1300 and 1800 on day 3 of the estrous cycle.

Fatty acid oxidation was inhibited by intragastric treatment with MP, a drug that binds irreversibly to carnitine palmitoyltransferase I, the enzyme necessary for transport of long-chain FFAs into mitochondria (37). MP (R. W. Johnson Pharmaceutical Research Institute) was suspended in 0.5% methyl cellulose and given by intragastric intubation at 20 mg/kg every 12 h, starting at 0800 on day 1 and ending at 2000 on day 2 of the estrous cycle. In previous studies, we found that estrous cycles of ad libitum-fed hamsters are not significantly inhibited by doses of MP ranging between 10 and 100 mg/kg (27). In the present study, we used doses of MP that were high enough to significantly inhibit FFA oxidation (31) but were not high enough to induce anestrus in ad libitum-fed Syrian hamsters (20 mg/kg) (27).

To inhibit glucose oxidation, we used 2DG (Sigma, St. Louis, MO), a glucose analog that competitively inhibits glucose oxidation at the phosphohexoseisomerase step. We used doses of 2DG that induce glucoprivation but do not induce anestrus in ad libitum-fed Syrian hamsters (1,000 mg/kg) (27). 2DG was dissolved in 0.9% saline and injected intraperitoneally every 8 h starting at 2200 on day 4 and ending at 2200 on day 2 of the estrous cycle.

Forty-nine estrous cycling hamsters between 90 and 95 g in body weight were divided into seven groups that did not differ significantly in body weight at the start of the experiment: 1) fasted-vehicle-vehicle (n = 5), 2) fasted-vehicle-leptin (n = 6), 3) fasted-MP-leptin (n = 11), 4) fasted-2DG-leptin (n = 6), 5) fasted-MP-vehicle (n = 9), 6) fasted-2DG-vehicle (n = 6), and 7) fed-vehicle-vehicle (n = 6). Any hamsters that did not receive leptin, 2DG, or MP received the appropriate vehicle. 2DG was injected every 8 h, three times per day, whereas MP was given every 12 h, twice per day. Treatments and fasting occurred on days 1 and 2 of the estrous cycle. Estrous behavior tests began 1 h before “lights out” on day 4 of the estrous cycle after the above treatments were completed. After the start of treatments, 5-min behavior tests were carried out in the female subjects’ home cage beginning 1 h before the onset of darkness (2100) on estrous cycle day 4. An adult male was introduced in the female’s home cage. Females that assumed the immobile posture accompanied by a rigid dorsoflexion of the spine characteristic of lordosis were scored positive for estrous behavior. If lordosis did not occur during the 5-min test, females were scored negative for estrous behavior. Tests were discontinued before the 5-min period if biting attacks from the female endangered the male. If behavior tests continued into the dark period, they were conducted under red illumination. If a female did not show lordosis on estrous cycle day 4, tests were continued at the same time each day until lordosis occurred. Tests for postovulatory vaginal discharge occurred on the morning of day 1 of the next expected cycle, between 4 and 12 h after the onset of the light period.

The frequencies of hamsters showing lordosis and vaginal discharge were analyzed by the row × column test of independence, a G test for goodness of fit. Estrous cycle length was analyzed by analysis of variance followed by Duncan’s multiple range test for post hoc comparisons when main effects were significant.

RESULTS

Intracerebroventricular leptin treatment reliably reversed the effects of fasting on estrous cyclicity (Figs. 1 and 2). None of the fasted-vehicle-vehicle hamsters showed normal 4-day estrous cycles, whereas 100% of the fasted-vehicle-leptin hamsters showed 4-day estrous cycles (P < 0.0001; Fig. 1). Fasted-vehicle-leptin hamsters had significantly shorter estrous cycle lengths than fasted-vehicle-vehicle hamsters (P < 0.01; Fig. 2).
In contrast, leptin did not reliably reverse fasting-induced anestrus in MP- or 2DG-treated hamsters. Only 40% of the fasted-MP-leptin and 20% of the fasted-2DG-leptin hamsters showed normal, 4-day estrous cycles. In fasted-MP-leptin and fasted-2DG-leptin groups, the number of hamsters showing 4-day estrous cycles and the estrous cycle length were not significantly different from the fasted-vehicle-vehicle group (Figs. 1 and 2). Fasted-2DG-leptin hamsters had significantly longer estrous cycle lengths than those of the fasted-vehicle-leptin hamsters ($P < 0.05$).

**DISCUSSION**

The primary finding was that intracerebroventricular leptin treatment reversed fasting-induced anestrus but failed to do so when metabolic fuel oxidation was inhibited (Figs. 1 and 2). The results provided unequivocal evidence that a high level of leptin in the central nervous system is an insufficient condition for normal estrous cyclicity when fuel oxidation is inhibited. Furthermore, the study demonstrates an interaction between the effects of leptin and the effects of 2DG and MP. It is possible that the interaction occurs at the level of intracellular fuel oxidation. In general, 2DG treatment decreases glucose uptake and oxidation, whereas leptin treatment increases glucose uptake and oxidation (22). MP treatment inhibits oxidation of long-chain FFA, whereas leptin treatment decreases triglyceride synthesis and increases levels of FFA oxidation in peripheral tissues (14, 16, 21, 36). At least two studies have confirmed that leptin treatment (either peripheral or intracerebroventricular) decreases food intake and at the same time prevents the increase in plasma ketone bodies characteristic of decreased food intake. This suggests that leptin treatment can increase FFA availability and oxidation in peripheral tissues without mobilization of FFAs (36, 38). Other studies have provided direct evidence that intracerebroventricular leptin treatment increases peripheral energy expenditure, glucose uptake, and lipid mobilization (21, 24). Together these data are consistent with the idea that estrous cyclicity is controlled by changes in fuel availability and oxidation and the idea that the reversal of fasting-induced infertility by exogenous leptin treatment is due to indirect effects of leptin on fuel availability and oxidation. Signals generated by changes in fuel oxidation might arise from brown adipose tissue, skeletal muscle, liver, gut, or pancreas, and these signals might feed back to the hypothalamic-pituitary-gonadal axis via peripheral afferents. Alternatively, anestrus might be caused by decreased fuel metabolism in ovary or pituitary. Furthermore, the interaction between leptin and fuel oxidation might occur in brain. It should be noted, however, that a central neuroendocrine interaction does not fully explain the interaction between leptin and MP. It is thought that MP does not reach the brain in appreciable quantities (37), and cells in the central nervous system primarily use glucose rather than FFAs. Thus any metabolic interaction between intracerebroventricular leptin and MP treatment is more likely to occur peripherally rather than centrally.

This study demonstrates an interaction between exogenous leptin treatment and fuel metabolism, but does not address the idea that naturally occurring endogenous fluctuations in leptin affect reproduction via metabolic or other mechanisms. It is not clear that naturally occurring endogenous increases in leptin, and leptin-induced increases in fuel metabolism are critical for normal estrous cyclicity in Syrian hamsters (Schneider et al., unpublished observations).

**Perspectives**

It has been demonstrated repeatedly that food intake and reproductive processes can be influenced by signals that are generated peripherally by either the oxidation of metabolic fuels or by signals that arise from digestive processes such as gut emptying. Other excellent work has implicated the caudal brain stem in either the detection and/or integration of signals that control food intake and reproductive processes. In addition, it has been demonstrated that areas of the hypothalamus also influence food intake and reproductive processes via their projections to peripheral sites that control energy expenditure, metabolic fuel oxidation, and digestive function. The cloning of the ob gene, the discovery of the effects of exogenous leptin treatment on food intake and reproduction, and the identification of leptin receptors in hypothalamus, brain stem, and periphery are all consistent with the metabolic hypothesis. Leptin, whether applied to sites in the brain or periphery,
produces changes in metabolic fuel oxidation, energy expenditure, and gut emptying that would be expected to decrease food intake and facilitate reproductive processes. It is unfortunate then that the most well-publicized models of leptin action portray leptin solely as an adipostatic signal that reflects body fat content and acts directly in the hypothalamus to control food intake and reproduction (6, 7, 10, 11, 19). These latter models are incomplete and possibly misleading because they fail to incorporate the idea of distributed (peripheral and central) metabolic and neuroendocrine control of food intake and reproduction.

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