Suppression and recovery of estrous behavior in Syrian hamsters after changes in metabolic fuel availability

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Jones, Juli E., and Laura S. Lubbers. Suppression and recovery of estrous behavior in Syrian hamsters after changes in metabolic fuel availability. Am J Physiol Regulatory Integrative Comp Physiol 280: R1393–R1398, 2001.—A reduction in the availability of oxidizable metabolic fuels inhibits reproduction. Forty-eight hours of metabolic fuel deprivation inhibits estrus behavior in ovariectomized, steroid-treated Syrian hamsters, but little is known about the time course of this inhibition. Likewise, refeeding reverses deprivation-induced suppression, but the rate of recovery has not been examined. In two experiments we determined 1) the rate at which estrous behavior declines in hamsters treated with metabolic inhibitors and 2) how rapidly sexual receptivity is restored when hamsters are refed after a 48-h fast. We also measured circulating levels of leptin and insulin in an attempt to determine their relationship to the inhibition and restoration of estrus behavior. More than 24 h of metabolic inhibitor administration were required to inhibit lordosis, whereas only 6 h of refeeding were sufficient to restore the display of sexual receptivity to normal levels. Neither plasma insulin nor leptin levels paralleled the changes in estrus behavior. We concluded that 1) suppression of estrous behavior occurs more slowly than recovery after a fast and 2) changes in circulating leptin and insulin probably do not have a critical role in these behavioral changes.

methyl palmitate; insulin; leptin; food deprivation; 2-deoxy-d-glucose

Adequate supplies of metabolic fuels are necessary to sustain all of life’s physiological processes, including reproduction. Because reproduction is exceedingly costly, especially for female mammals, it is closely regulated by the availability of metabolic fuels. This becomes apparent when food is scarce, when an animal’s ability to utilize metabolic fuels is inhibited, or when energy expenditure exceeds intake. Under these conditions reproduction is interrupted (3, 17, 25, 32).

Syrian hamsters have become a useful model for studying the metabolic requirements for reproduction. Syrian hamster estrous cycles are highly sensitive to food deprivation; just 48 h of food deprivation on days 1 and 2 (estrus and diestrus [I]) of the cycle is sufficient to inhibit ovulation and estrous behavior (21). Estrous behavior also is suppressed in steroid-primed ovariec-
al. (10), we found that normal levels of lordosis were found in hamsters that were refed for 24 or 48 h (data not shown). Hence, a further investigation using time points between 0 and 24 h of refeeding was conducted.

Leptin and insulin appear to be involved in the control of reproduction (1). Circulating levels of leptin and insulin vary in proportion to the amount of adipose tissue, and it has been suggested that circulating levels of these two metabolic hormones may signal the availability of metabolic fuels to the neural circuits controlling reproduction (1, 12, 16, but cf. 29). Another aim of these experiments was to determine physiological correlates of the metabolic inhibitor-induced suppression of lordosis and the refeeding-induced restoration of lordosis, beginning with circulating levels of leptin and insulin. If these hormones play a crucial role in the suppression and recovery of estrous behavior, then the changes in their levels should precede the fluctuations in lordosis.

MATERIALS AND METHODS

Animals and housing. One hundred ten female Syrian hamsters (*Mesocricetus auratus*) weighing 80–100 g were obtained from Charles River Breeding Laboratories (St. Constant, Quebec). Animals were singly housed in wire-bottom, stainless steel cages (17.5 × 17.5 × 17.5 cm) in a room maintained at 22°C with a 14:10-h light-dark cycle (lights on at 0700). Hamsters were fed Purina Laboratory Chow (no. 5001) placed in hoppers mounted on the outside of the cages. Food and water were available ad libitum except when indicated. All procedures were approved by the University of Massachusetts Institutional Animal Care and Use Committee.

Surgery and behavioral testing. After a 1-wk period of adaptation to the laboratory, hamsters were anesthetized using pentobarbital sodium (80 mg/kg), supplemented when necessary with Metofane (methoxyflurane; Pitman-Moore, Mundelein, IL), and bilaterally ovariectomized. Two weeks following surgery, hamsters were primed with estradiol benzoate (EB, 2.5 µg sc, dissolved in 0.1 ml sesame oil) followed 42 h later with progesterone (500 µg sc, dissolved in 5% benzyl alcohol and 15% benzyl benzoate in 0.1 ml sesame oil) (8, 10, 20, 24, 31). Two weeks after this priming regimen, all hamsters were given the same steroid treatment. Six hours after progesterone injection, the animals were placed with males and screened for sexual receptivity.

Behavioral testing was conducted as follows. Each hamster was placed alone in a Plexiglas arena (30 × 36 × 30 cm) for 5 min. After the 5-min habituation to the testing chamber, a sexually experienced male hamster was placed in the test chamber with the female for 3 min while the experimenter continually brushed the female’s flanks with an artist’s paintbrush to ensure that she received consistent tactile stimulation (24). The male was permitted to investigate and mount the female but not to intromit. During this time, the amount of time that the female spent in lordosis was recorded. The animals that did not display lordosis were removed from the study. The remaining animals were randomly placed into treatment groups, counterbalanced for body weight.

Experiment 1. Two weeks after the screening test, 72 animals were given the same steroid-priming regimen before behavioral testing. The treatment groups were given metabolic inhibitors 0, 3, 6, 12, 24, and 48 h before the behavioral test (Fig. 1). The inhibitor-treated animals were given 2-DG (750 mg/kg ip, in 0.15 M NaCl) and MP via gavage (25 mg/kg suspended in 0.5% methyl cellulose). At the time points when animals did not receive metabolic inhibitors, they were given both vehicles (0.15 M NaCl and 0.5% methyl cellulose). All animals were allowed to eat ad libitum. At the time of EB injection, all animals began to receive both intraperitoneal (vehicle or 2-DG) and gavage (vehicle or MP) treatments every 6 h until behavioral testing. Administration of MP began 6 h before the onset of 2-DG administration. Immediately after behavioral testing, animals were killed by decapitation. Trunk blood was collected and centrifuged, and plasma was frozen (−22°C) until time of hormone assay.

Experiment 2. Two weeks after a behavioral screening test, a different set of 60 animals was given the same steroid-priming regimen as in the first experiment. One group of hamsters was fed ad libitum throughout the experiment, whereas the other five groups were fasted for 48 h and refed for either 0, 3, 6, 12, or 24 h prior to the behavioral test (Fig. 1). Immediately after behavioral testing, animals were killed by decapitation and trunk blood was collected and processed as in the first experiment.

Hormone assays. Plasma leptin levels were measured using the Linco Multi-Species Leptin RIA kit, XL-85K (St. Charles, MO). The leptin lower-detection limit was 2.0 ng/ml. Plasma insulin levels were measured using the Linco Rat Insulin RIA kit, FI-13K. The insulin lower-detection limit was 0.1 ng/ml.

Data analysis. All statistical analyses were carried out by one-way ANOVA. Significant differences at α = 0.05 were followed with Fisher’s least significant difference post hoc analysis.
RESULTS

Experiment 1: Suppression of lordosis. Forty-eight hours of metabolic inhibitor administration significantly reduced lordosis duration compared with animals given metabolic inhibitors for 0, 3, or 6 h, but not compared with animals that received treatments for 12 and 24 h \( F(5,65) = 2.43, P < 0.05 \); yet the animals that received metabolic inhibitors for 12 or 24 h were not significantly different from those that received inhibitors for 0, 3, or 6 h (Fig. 2). Metabolic inhibitor administration did not significantly affect either leptin \( F(5,26) = 1.28, P > 0.3 \) or insulin levels \( F(5,62) < 1 \); Fig. 3).

Experiment 2: Recovery of lordosis. Lordosis duration was significantly reduced following 48 h of food deprivation in EB- plus progesterone-treated hamsters. Lordosis duration remained suppressed after 3 h of refeeding. However, lordosis duration was not different from ad libitum-fed hamsters following 6, 12, and 24 h of refeeding \( F(5,54) = 16.48, P < 0.001 \); Fig. 4). Plasma leptin \( F(5,54) = 4.14, P < 0.01 \) and insulin \( F(5,54) = 8.8, P < 0.001 \) levels were significantly reduced by 48 h fast. Insulin remained significantly reduced at all subsequent time points. The same was true of leptin except at the 6-h refeeding time point (Fig. 5).

DISCUSSION

The results of these experiments indicate that 1) more than 24 h of metabolic inhibitor administration is necessary to suppress estrous behavior, 2) 6 h of refeeding is sufficient to restore estrous behavior after a 48-h fast, and 3) neither circulating leptin nor insulin levels are correlated with changes in expression of estrous behavior under these conditions.

More than 24 h of metabolic fuel restriction, whether due to food deprivation or metabolic inhibitor administration, was required to significantly suppress lordosis. This would suggest that the long latency for food deprivation-induced suppression of lordosis is not due solely to a slowly developing metabolic cue. In monkeys, rats, and sheep, the metabolic consequences of...
2-DG and MP treatment affect LH pulsatility very quickly (5, 7, 22), but for hamsters more than 24 h of treatment is still required for the suppression of estrous behavior (Fig. 2). These results indicate that the suppression of lordosis develops more slowly than the inhibition of pulsatile LH release. Thus it appears that the neural mechanisms controlling GnRH (2) respond to metabolic cues much more rapidly than those controlling lordosis or else they respond to different cues, which make this process more rapid. Indeed, a number of neuropeptides that affect food intake, including neuropeptide Y (8) and corticotropin-releasing hormone (Jones, unpublished data) can suppress lordosis within minutes in ad libitum-fed, steroid-primed hamsters. Thus the delay in suppression of estrous behavior may lie in the very gradual processing of information somewhere between detection of the metabolic signals and the release of inhibitory neurotransmitters and/or neuropeptides.

In contrast to inhibition of estrous behavior, recovery is more rapid. Our results clearly demonstrate that estrous behavior recovers rapidly and abruptly following refeeding after a 48-h fast in female Syrian hamsters. There was no evidence of recovery by 3 h of refeeding, but by 6 h lordosis duration had returned to normal levels. This indicates that metabolic cues related to refeeding are detected and processed relatively quickly. This 3- to 6-h latency to recovery is still somewhat slower than the latency reported for resumption of LH pulses in other species (typically 1–2 h) (4, 13, 23). It is not clear whether this reflects differences in the neuroendocrine mechanisms controlling estrous behavior vs. pulsatile LH release. Another possibility is that the longer latency for restoration of estrous behavior could be due to the absence of a postfast hyperphagia in Syrian hamsters (30) in contrast to other species, which were used to study LH pulsatility (4, 23).

The fact that estrous behavior recovers rapidly in refeed hamsters suggests that the physiological processes contributing to reinstatement of estrous behavior in food-deprived animals also respond rapidly and likely before restoration of body lipid or glycogen stores. Thus rapid recovery of estrous behavior after a fast gives a relatively narrow temporal window in which to look for relevant neuroendocrine changes.

Circulating levels of leptin and insulin vary in proportion to the amount of adipose tissue in the body, and it has been suggested that circulating levels of these two metabolic hormones may signal the availability of metabolic fuels to the neural circuits controlling reproduction (1, 12, 16, but cf. 29). Receptors for leptin and insulin are found in the central nervous system and other tissues and have a role in the regulation of energy balance (16, 18). Animals with mutations of either the leptin or leptin receptor genes are obese and infertile. Treatment of leptin-deficient animals with exogenous leptin causes weight loss and restores some indexes of fertility (11, 28). In wild-type animals, leptin treatment can prevent some of the suppressive effects of food deprivation on reproduction. For example, in Syrian hamsters, administration of leptin reverses the effects of food deprivation on estrous cyclicity, but it does not restore reproductive behaviors in food-deprived, ovariectomized animals treated with exogenous steroids (26, 31). In fact, treatment with leptin intensified the inhibition of estrous behavior in food-deprived hamsters, whereas it facilitated female sexual behavior in ad libitum-fed hamsters (31).

Adequate levels of insulin also are required for normal fertility. Streptozotocin-treated diabetic female rats and hamsters are typically anovulatory and exhibit reduced behavioral responsiveness following treatment with ovarian steroids (15, 20). These deficits are corrected by insulin replacement (19, 20). Therefore, the presence of some level of both leptin and insulin is required for normal estrous behavior. However, it is not at all clear whether fluctuations in circulating levels of these two hormones within the normal physiological range play a crucial role in the control of reproduction by food availability.

In the present experiments, circulating levels of leptin and insulin bore no consistent relation to levels of estrous behavior. Neither plasma leptin nor insulin levels decreased significantly with administration of metabolic inhibitors. Moreover, plasma leptin and in-
sulin levels were not restored prior to recovery of estrous behavior. In fact, leptin and insulin levels remained low even after 24 h of refeeding, long after estrous behavior was restored. Thus while the presence of some insulin and leptin is required for normal fertility in a number of species, maintenance of fed levels of these two hormones does not appear to be required for the expression of normal levels of estrous behavior in Syrian hamsters. These findings are consistent with previously published work in both rats and hamsters (14, 31). However, these findings do not preclude a significant role for leptin and/or insulin signaling in the control of lordosis via changes in leptin and/or insulin neural receptor abundance.

During metabolic fuel deprivation there are myriad changes in neuroendocrine and/or metabolic function, and it is often difficult to determination the existence and direction of causality among these events. The gradual suppression of estrous behavior and its more rapid recovery following manipulations of metabolic fuel availability provide a framework within which to study the neuroendocrine bases of the behavioral aspects of nutritional infertility. Along with previous work on the time course of changes in LH secretion, this information will aid in determining which physiological events play a causal role in altering hormone secretion and estrous behavior. This step in that direction does not provide any support for a crucial role for physiological fluctuations in either insulin or leptin levels.

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