

Manipulations of Metabolic Fuel Availability Alter Estrous Behavior and Neural Estrogen Receptor Immunoreactivity in Syrian Hamsters*

HUI-YUN LI†, GEORGE N. WADE, AND JEFFREY D. BLAUSTEIN

*Neuroscience and Behavior Program and Department of Psychology,
University of Massachusetts, Amherst, Massachusetts 01003-7710*

ABSTRACT: Decreases in metabolic fuel utilization caused by food deprivation, diabetes, or treatment with metabolic inhibitors have been shown to suppress steroid-induced estrous behavior in ovariectomized Syrian hamsters. These same manipulations also caused a decrease in the number of detectable estrogen-receptor immunoreactive (ERIR) cells in the ventromedial hypothalamus (VMH) and the adjacent area lateral to it (VLH) in ovariectomized hamsters. Forty-eight h of food deprivation or treatment with pharmacological blockers of glycolysis (2-deoxy-D-glucose) and fatty acid oxidation (methyl palmoxirate) decreased the number of detectable ERIR cells in the VMH/VLH. However, neither inhibitor given alone was sufficient to affect the number of detectable ERIR cells in the VMH/VLH, even when given in much higher doses. Therefore, the number of ERIR cells in the VMH/VLH, like steroid-induced estrous behavior, responds only to a combination of glucoprivation and lipoprivation and not to either condition alone. The effects of metabolic fuel restriction are not due to a general suppression of neural ERIR, because food deprivation or treatment with 2-deoxy-D-glucose + methyl palmoxirate actually increased the number of detectable ERIR cells in the medial preoptic area (mPOA) and had no effect in the nucleus of the solitary tract. Lesions destroying the area postrema (AP) prevented the decrease in ERIR cells in the VMH/VLH and the inhibition of estrous behavior caused by fuel restriction. However, AP lesions did not alter the effects of metabolic inhibitors on ERIR in the mPOA. On the other hand, subdiaphragmatic vagotomy abolished the effects of metabolic inhibitors on mPOA ERIR but did not affect either lordosis or VMH/VLH ERIR.

These results suggest that the suppression of estrous behavior induced by metabolic fuel restriction is at least in part due to a decrease in the number of ERIR neurons in the VMH/VLH. In addition, the estrogen-sensitive neurons in the VMH/VLH and mPOA receive metabolic cues via different neural pathways. The AP, but not vagus nerves, is required for ERIR neurons in the VMH/VLH to detect fuel availability; the vagus nerves, but not the AP, are necessary for estrogen-binding neurons in the mPOA to detect visceral information. *Endocrinology* **135**: 240-247, 1994.

FOOD AVAILABILITY is the single most important environmental factor governing mammalian reproduction (1). In female mammals, reproduction is energetically costly and highly sensitive to the food supply. Fuel restriction delays puberty or interrupts ovulatory cycles in rodents (1-3), domestic animals (1, 4, 5) and primates (6, 7). Metabolic manipulations that interrupt estrous cycles also affect behaviors, including a suppression of estrous behavior (8). For example, in Syrian hamsters, just 48 h of food deprivation interrupts estrous cycles (9) and suppresses steroid-induced estrous behavior in ovariectomized animals (8).

The effect of food deprivation on hamster estrous cycles is mimicked by an inhibition of glycolysis (glucoprivation) using treatment with 2-deoxy-D-glucose (2DG). On the other hand, inhibition of fatty acid oxidation (lipoprivation) using methyl palmoxirate (MP) induces a hibernation-like torpor in many hamsters without affecting estrous cycles in the animals that remain euthermic (10). In ovariectomized animals, steroid-induced estrous behavior is suppressed by a combination of glucoprivation and lipoprivation, but not by either metabolic inhibitor given alone, even in very high doses

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Address all correspondence and requests for reprints to: George N. Wade, Department of Psychology, Box 37710, University of Massachusetts, Amherst, Massachusetts 01003-7710.

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†Present address: The Salk Institute, P.O. Box 85800, San Diego, California 92186-5800

(8). Thus, in Syrian hamsters, these three responses to food deprivation (anovulation, torpor, and suppression of estrous behavior) appear to respond to different metabolic cues.

Although a large body of research has shown that mammalian reproductive physiology and behavior are sensitive to the availability of metabolic fuels, very little is known about the neural mechanisms involved in nutritional regulation of fertility. Metabolic fuel availability can act at all levels of hypothalamic-pituitary-ovarian axis, but the primary locus of nutritional control of ovulatory cycles and pituitary hormone secretion in females appears to be GnRH secretion by forebrain neurons (1, 3, 11). The fact that manipulations of metabolic fuel availability that interrupt ovulatory cycles in hamsters also suppress the expression of *Fos* immunoreactivity in GnRH neurons supports the hypothesis that nutritional infertility is due in part to a decrease in GnRH secretion (12).

It is possible that the fuel-deprivation-induced decrease in behavioral responsiveness to exogenous ovarian steroids is due to a disruption of estradiol and/or progesterone binding in the brain. Ovarian steroids act via neural estrogen and progestin receptors to alter neuronal function and, consequently, behaviors (13, 14). Disturbances of metabolic fuel metabolism caused by acute withdrawal of exogenous insulin significantly impair estradiol and progesterone-induced estrous behavior in ovariectomized, streptozotocin-induced diabetic rats (15-17). This impairment of steroid-induced estrous behavior is accompanied by decreased *in vivo* cell nuclear binding of estradiol in grossly dissected hypothalamus and preoptic area (15-17). These findings are consistent with the possibility that metabolic fuel availability influences estrous behavior by altering neural steroid binding. However, no one has actually reported the effects of food deprivation or glucoprivation/lipoprivation, which suppress hamster estrous behavior, on neuronal steroid receptors; nor has anyone applied any higher-resolution neuroanatomical procedures to this problem in any species.

In the following experiments, we first use a recently developed immunocytochemical technique for detection of estrogen-receptor immunoreactivity (ERIR) in Syrian hamster brain (18) to look at the effects of manipulations of metabolic fuel availability on ERIR in the medial preoptic area (mPOA), the ventromedial hypothalamus (VMH) and the area just lateral to it (VLH), and the nucleus of the solitary tract (NTS). We chose these sites for our initial studies, because they contain high concentrations of estrogen receptors and play an important role in the behavioral and physiological actions of estradiol (VMH and mPOA) (13, 14, 18-22) or because they contain estrogen receptors (Li, unpublished results) and participate in the central processing of visceral sensory information (NTS) (23). In addition, to permit comparisons with earlier work with diabetic rats, we examined the effects of diabetes mellitus on estrous behavior and neural ERIR in hamsters. The results of these experiments indicate that manipulations of metabolic fuel availability do, indeed, alter the number of detectable ERIR neurons in the VMH/VLH and mPOA, but they provide no information as to which neural pathways detect and transmit metabolic fuel information to these forebrain ERIR neurons.

Prior research on how and where information about metabolic fuel availability is monitored and transmitted to the neural circuitry that mediates feeding behavior provides a useful framework for studies of the metabolic controls of estrous behavior. Signals generated in both glucose and fatty acid oxidation participate in the control of food intake; increases in food intake are found in rats treated with inhibitors of either glycolysis or fatty acid oxidation (23-25). Several areas in the hindbrain play roles in conveying metabolic fuel information to the forebrain. The NTS is the major visceral relay cell group in the brain (26). Some of these inputs come from autonomic sensory nerves, and others come from the area postrema (AP), a circumventricular organ situated in the floor of fourth ventricle that functions as a sensor of the chemical environment (*i.e.* plasma and cerebrospinal fluid) (27) and transmits this information to other areas of the brain. The vagus nerves convey visceral information from the gut, liver, and cardiorespiratory system, and the NTS is the primary site of the termination of general visceral afferents in the vagus nerve (28). The NTS in turn has extensive efferent projections to the lateral parabrachial nucleus, which is the major relay from the AP/NTS to the forebrain (29). There are also direct projections from the NTS to the forebrain (30).

Ablation of the AP/NTS and subdiaphragmatic vagotomy have been widely employed for analyzing autonomic mechanisms participating in gastrointestinal physiology, behavior, and energy metabolism. In rats, lipoprivic feeding is abolished by vagotomy or lesions of the AP/NTS (31). This implies that lipoprivation is detected peripherally. In contrast, glucoprivic feeding is abolished by AP/NTS lesions, but not by vagotomy, suggesting that glucoprivation is detected centrally (and perhaps peripherally). Therefore, it is possible that glucoprivic and lipoprivic feeding are mediated by distinct metabolic detectors and neural pathways (23).

The last two experiments were designed to assess the roles of the AP and the vagus nerve in transmission of metabolic signals to the ERIR neurons in the forebrain that are involved in the expression of estrous behavior and perhaps other estrogen-sensitive endpoints.

Materials and Methods

Animals and housing

Female Lak:LVG Syrian hamsters weighing 90-100 g were purchased from Charles River Breeding Laboratories (Wilmington, MA) and housed in stainless-steel, wire-bottom cages ($17.5 \times 17.5 \times 25$ cm) at 22 ± 2 C. Hamsters were housed in a long photoperiod (LD 14:10; lights on at 0500 h) and given *ad libitum* access to Purina Laboratory Rodent Chow (no. 5001, Ralston-Purina, St. Louis, MO) pellets and tap water. Two to 6 wk prior to each experiment, animals were ovariectomized through bilateral flank incisions under sodium pentobarbital anesthesia (80 mg/kg, ip).

Immunocytochemistry

ERIR was assessed by an immunocytochemical technique that has been described and validated previously (18). Hamsters were anesthetized with sodium pentobarbital (80 mg/kg, ip). Five thousand units of sodium heparin dissolved in 1.0 ml of 0.15 M sodium chloride were injected directly into the left ventricle followed by cutting the right atrium of the heart. A cannula was inserted into the left ventricle. Sodium chloride (75-100 ml) preceded the flow of fixative (2% paraformaldehyde in 0.1 M sodium phosphate buffer containing 0.4% glutaraldehyde). Perfusion pressure and flow rate were maintained at 100 mmHg and 25 ml/min, respectively, for 10 min. The brain was removed from the cranium and stored at 4 C overnight in 0.1 M sodium phosphate containing 20% sucrose. In *Exp 1*, 40 μ m coronal sections were cut through the preoptic area and hypothalamus as well as hindbrain on a freezing microtome and stored in cryoprotectant at -20 C. In *Exp 2-4*, only forebrain was cut and stored. Free-floating sections were rinsed three times for 5 min each with 0.05 M Tris-buffered saline (TBS; pH 7.6) and 1% sodium borohydride (in TBS) for 10 min to reduce residual aldehydes. Sections were then rinsed four times with TBS followed by incubation for 20 min in TBS with 1% hydrogen peroxide, 1% bovine serum albumin, and 20% normal rabbit serum to deplete endogenous peroxidase activity that may interfere with specific staining. The sections were incubated in primary antibody (H222; Abbott Laboratories, North Chicago, IL) at a concentration of 1 μ g/ml in TBS containing 0.5% Triton X-100, 0.1% gelatin, and 0.02% sodium azide, pH 7.6 at 4 C for 72 h. Sections were then rinsed with the same buffer three times for 5 min each followed by incubation in secondary antiserum (biotinylated rabbit anti-rat immunoglobulin, Vector Laboratories, Burlingame, CA), diluted in the same buffer as the primary antibody at a concentration of 6 μ g/ml at room temperature for 90 min. Following two rinses with and one without Triton X-100 (5 min each), the sections were incubated for another 90 min in the avidin DH: biotinylated horseradish peroxidase H complex (Vectastain Elite ABC kit, Vector Laboratories) diluted at a concentration of 1:100 in TBS. Following washing with TBS containing 0.5% Triton X-100 and 0.02% sodium azide at pH 7.6 two times and TBS once, sections were reacted with diaminobenzidine (0.05% in TBS) for 10 min in the presence of hydrogen peroxide (0.05%) and rinsed in TBS.

We have shown previously (18) that the H222 monoclonal antibody can be used to detect ERIR cells in several areas in hamster brain, including the preoptic area, mediobasal hypothalamus and amygdala. Furthermore, in guinea pigs (32) and in hamsters (33), preadsorption of the H222 antibody with estrogen receptors eliminates ERIR.

In *Exp 1*, the number of ERIR cells was determined in the VMH/VLH, mPOA and NTS. In *Exp 2*, only the VMH/VLH was examined; in *Exp 3* and *4* the VMH/VLH and mPOA were examined. ERIR cells were counted unilaterally in the mPOA and VMH/VLH, and bilaterally in the NTS. The mPOA consisted the anterior aspect of the mPOA, caudal to the crossing of the anterior commissure (Fig. 1A). The VMH/VLH sections were counted at the level of caudal median eminence and rostral to the formation of the infundibulum. ERIR cells were counted in both the ventromedial nucleus (VMN) proper and in the area immediately lateral to it (VLH) (Fig. 1B) that also contains ERIR cells (18). The NTS was examined at the level of the pyramidal decussation and immediately caudal to the area postrema (Fig. 1C). Two adjacent sections were chosen from each

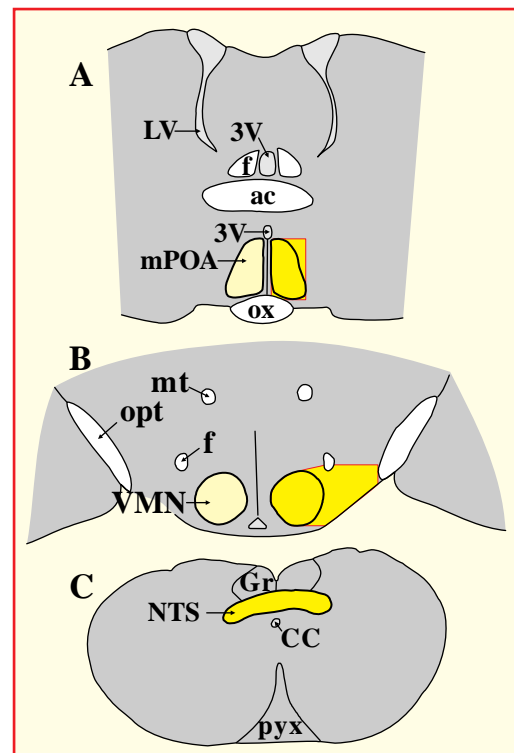


FIG. 1. Schematic drawing of locations where ERIR cells were counted (*hatched areas*). mPOA, medial preoptic area; VMN, ventromedial nucleus of the hypothalamus; NTS, nucleus of the solitary tract; 3V, third ventricle; LV, lateral ventricle; f, fornix; ac, anterior commissure; ox, optic chiasm; mt, mammillothalamic tract; opt, optic tract; CC, central canal; Gr, gracile nucleus; pyx, pyramidal decussation.

area by carefully matching their landmarks under dark field illumination. Sections were matched across all animals in all experimental groups before counting began. The experimenter was blind as to treatment groups during matching. ERIR cells in the mPOA were counted using computer-aided image analysis (Image 1.47, W. Rasband, National Institutes of Health). At the beginning of the analysis of the first sample, the camera gain level and black level were adjusted at optimal levels which gave a normal distribution of gray levels falling within the parameter of the imaging system: that is 0 to 254 gray scales. This light level was maintained throughout the analysis in order to obtain consistent measurements. Due to the background generated during immunocytochemical processing, it was difficult to count ERIR cells in the VMH/VLH and NTS using this imaging system. ERIR cells in the VMH/VLH and NTS were counted under a light microscope; the criterion for localization of ERIR cells was the presence of an immunostained cell nucleus. The experimenter was always blind as to the treatment groups of the sections being counted.

Procedures

Exp 1: food deprivation and metabolic inhibitors. Ovariectomized hamsters were fed *ad libitum* (n = 4) or deprived of food (n = 4) for 48 h prior to perfusion. A third group of hamsters (n = 3) was fed *ad libitum* and treated with 2DG (750 mg/kg, ip) and MP (25 mg/kg by gavage) once every 6 h for 48 h prior to perfusion. The fourth group (n = 4) was fed *ad libitum* and treated with the respective vehicles (saline for 2DG and 0.5% methyl cellulose for MP). Forty-eight h of food deprivation or treatment with these doses of 2DG + MP is sufficient to suppress steroid-induced estrous behavior in ovariectomized hamsters (8).

This experiment was then repeated with *ad libitum*-fed, ovariectomized hamsters treated with vehicles (n = 4), a high dose of 2DG (1750 mg/kg; n = 3), or a high dose of MP (75 mg/kg; n = 3) once every 6 h for 48 h prior to perfusion. This dose of 2DG is sufficient to interrupt estrous cycles, and this dose of MP is sufficient to induce hibernation-like torpor in gonadally intact hamsters (10). However, neither treatment suppresses steroid-induced estrous behavior in ovariectomized hamsters (8).

Exp 2: diabetes mellitus. Diabetes mellitus was induced in ovariectomized hamsters (n = 22) with 3 daily injections of streptozotocin (60 mg/kg, Sigma Chemical Co., St. Louis, MO) in citrate buffer (pH 4.8) (15). This dose of streptozotocin causes a rapid weight loss, hyperglycemia, and glycosuria (+++, TesTape, Eli Lilly Co., Indianapolis, IN). Control animals (n = 8) were injected with the citrate buffer vehicle. Diabetic hamsters were left untreated (n = 7) or were given 10 U of U-100 Lente insulin (Eli Lilly Co.) daily at 1000 h (n = 15). The insulin-treated, diabetic hamsters either continued to receive insulin replacement throughout the experiment (n = 8) or had insulin treatment withdrawn 58 h prior to the behavioral test (n = 7). Two wk after induction of diabetes, all animals were tested for estrous behavior. Lordosis was induced with a subcutaneous injection of 5 µg estradiol benzoate in 0.1 ml sesame oil at 1600 h followed 48 h later by a subcutaneous injection of 200 µg progesterone in 0.1 ml sesame oil. Four h after progesterone injection and 1 h before lights-out, animals were tested for lordosis which was quantified by recording the number of seconds spent in the lordosis posture out of 5 min in the presence of a male hamster in the female's cage. To provide a consistent level of flank stimulation to elicit lordosis, a pen brush was applied to female's flank region during the test period (34).

In the second part of this experiment, neural ERIR was measured in ovariectomized, diabetic hamsters 2 wk after treatment with streptozotocin (n = 4) and in nondiabetic animals that were fed *ad libitum* (n = 5) or food deprived for 48 h (n = 5).

Exp 3: AP lesions. Two wk after ovariectomy, animals were anesthetized with sodium pentobarbital (80 mg/kg, ip) and positioned in a stereotaxic instrument with the neck flexed to tilt the nose down. The skin and capitis musculature from the occipital crest to the midcervical level were dissected to expose the occipito-atlantal ligament, underlying dura mater, and arachnoid membrane. These structures were incised with forceps, and the occipital foramen magnum was visualized. After removal of the overlying meninges, the fourth ventricle was exposed, and the AP was aspirated with a 5-µl Wiretrol (Drummond, Broomall, PA). Sham AP lesions were done by exposing the dorsal hindbrain.

Ovariectomized, AP-lesioned and sham-operated animals were treated with either a combination of 2DG (750 mg/kg, ip) and MP (25 mg/kg by gavage) (n = 13 for lesioned group, n = 10 for control group) or vehicle (n = 13 for lesioned group, n = 10 for control group) every 6 h for 48 h prior to 3-min behavioral tests with males and flank stimulation. Two wk after the behavioral test, ERIR cells in the mPOA and VMH/VLH were counted (n = 5 in each group) after treatment with metabolic inhibitors or vehicles for 48 h as described above. Groups were balanced for prior treatment.

To confirm the placement of the lesions, animals were perfused as described above, and the medulla was removed and stored in 0.1 M sodium phosphate buffer containing 20% sucrose overnight. Forty µm coronal sections were cut at the level caudal of the fourth ventricle and rostral to the pyramidal decussation. Adjacent sections were placed onto gelatin-coated microscope slides and stained with cresyl violet. AP-lesioned sections then were examined with a light microscope to confirm the success of the aspiration lesions. Animals with incomplete lesions of the AP were omitted from the data analyses.

Exp 4: vagotomy. Two wk after ovariectomy, animals were anesthetized with sodium pentobarbital (80 mg/kg, ip). The abdomen was then opened with a midline incision. The liver lobes were retracted, and the stomach was gently moved to expose the esophagus. Total vagotomy (35) involved isolation of the branches and trunks of the vagus from the esophagus, omentum, and peritoneal fat. The nerve bundles of the anterior and posterior trunks were tied individually with surgical silk and sectioned between the stomach and the diaphragm.

Ovariectomized, vagotomized and sham-operated animals were treated with either a combination of 2DG (750 mg/kg, ip) and MP (25 mg/kg by gavage) (n = 14 for vagotomized group, n = 12 for control group) or vehicles (n = 13 for vagotomized group, n = 12 for control group) every 6 h for 48 h prior to 3-min behavioral tests with males and flank stimulation. Two wk after the behavioral

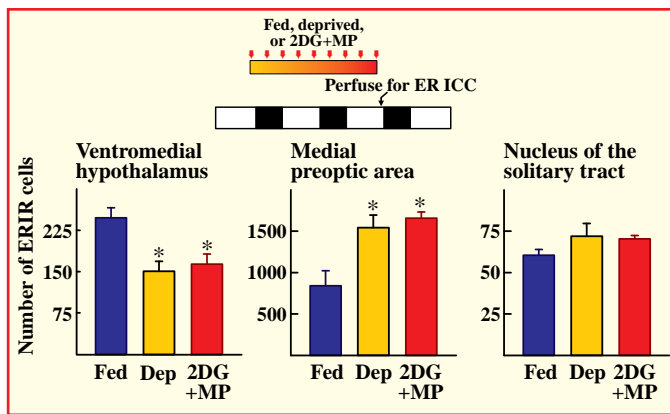


FIG. 2. Effect of *ad libitum* feeding, 48 h of food deprivation, or treatment 2DG (750 mg/kg) and MP (25 mg/kg) once every 6 h for 48 h on the number of detectable neurons containing ERIR in the VMH/VLH, mPOA, and NTS in ovariectomized hamsters. *, $P < 0.05$ vs. *ad lib.*-fed, vehicle-treated controls.

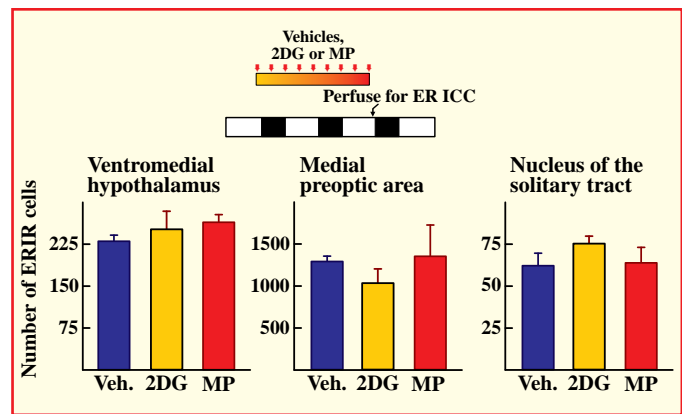


FIG. 3. Effect of treatment with vehicles, 2DG (1750 mg/kg) or MP (75 mg/kg) once every 6 h for 48 h on the number of detectable ERIR-containing neurons in the VMH/VLH, mPOA, and NTS in ovariectomized hamsters.

test, ERIR cells in the mPOA and VMH/VLH were counted ($n = 5$ in each group) after treatment with metabolic inhibitors or vehicles for 48 h as described above. Groups were balanced for prior treatment.

Completeness of vagotomy was determined by examining retrograde transport of fluorogold to the dorsal motor nucleus of the vagus following intraperitoneal injection (36). Three days prior to perfusion, animals were injected intraperitoneally with 0.5 mg of 4% fluorogold retrograde tracer (Fluorochrome, Inc., Englewood, CO). Animals were then perfused as described above. The medulla was removed and stored at 4 C overnight in 0.1 M sodium phosphate buffer containing 20% sucrose. Forty μ m coronal sections were cut between the level of the NTS and the pyramidal decussation. Serial sections were placed directly onto coated microscope slides and air dried. All sections were examined for fluorescent cells in the dorsal motor nucleus of the vagus under UV excitation light. The AP was checked for blood-borne labeling with fluorogold fluorescence to confirm the success of fluorogold injections. Sham-operated animals exhibited heavy fluorogold labeling in the dorsal motor nucleus of the vagus. Vagotomized animals with fluorogold in the dorsal motor nucleus of the vagus were omitted from data analyses.

Data analyses

Data were analyzed using one-way analyses of variance. In *Exp 1-2*, significant treatment effects were further analyzed by Newman-Keuls *post hoc* tests. In *Exp 3-4*, significant effects were further analyzed by pairwise *t*-tests or Mann-Whitney *post hoc* tests. Values were considered statistically significant when $P < 0.05$. Treatment with the saline and methyl cellulose vehicles had no effect on detectable neural ERIR, so the vehicle-treated and untreated control groups were combined for statistical analyses in *Exp 1*.

Results

Exp 1: food deprivation and metabolic inhibitors

Either 48 h of food deprivation or treatment with metabolic inhibitors caused a significant decrease in the number of detectable ERIR neurons in both VMH and the adjacent area lateral to it (VLH) (Fig. 2). These two areas responded identically to food deprivation and treatment with 2DG + MP. Therefore, they have been combined for analyses in the subsequent experiments.

In contrast, either 48 h food deprivation or treatment with 2DG + MP caused a significant increase in the number of detectable ERIR neurons in the mPOA (Fig. 2). Neither food deprivation nor treatment with 2DG + MP had any effect on ERIR in the NTS (Fig. 2). When given alone, even high doses of 2DG or MP had no effect on the number of ERIR neurons observed in the VMH/VLH, mPOA, or NTS (Fig. 3).

Exp 2: diabetes mellitus

As in rats (15-17), untreated diabetes mellitus significantly inhibited steroid-induced estrous behavior in ovariectomized hamsters, and continuous insulin replacement therapy prevented this inhibition of lordosis (Fig. 4). Insulin withdrawal 58 h prior to testing was also sufficient to cause a significant decrease in sexual receptivity. Either 2 wk of untreated diabetes or 48 h of food deprivation caused a significant decrease in detectable ERIR neurons in the VMH/VLH

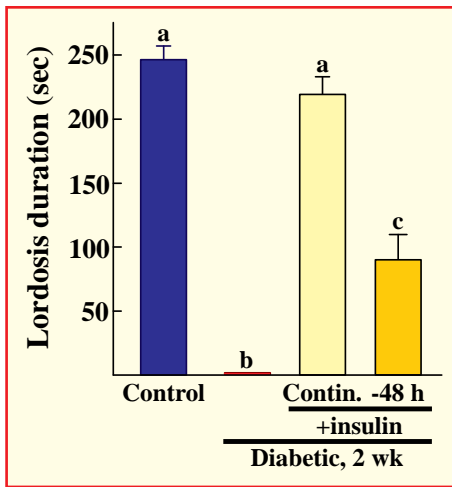


FIG. 4. Effect of streptozotocin-induced diabetes on estradiol + progesterone-induced estrous behavior in ovariectomized hamsters. Diabetic hamsters received either no insulin treatment, daily treatment of 10 U of Lente insulin throughout the experiment (Contin.), or daily treatment with 10 U of Lente insulin until 58 h prior to behavioral testing (-48 h). Bars with different letters are significantly different from each other ($P < 0.05$).

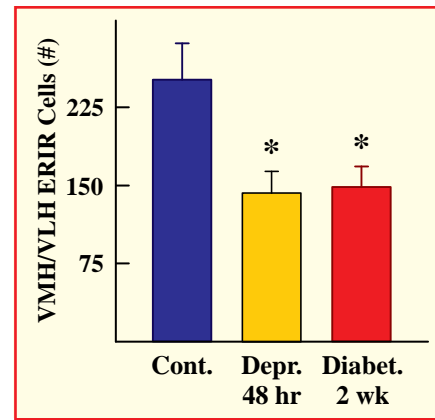


FIG. 5. Effect of 48 h food deprivation (Depr.) or 2 wk of streptozotocin-induced diabetes (Diabet.) on the number of detectable ERIR-containing neurons in the VMH/VLH of ovariectomized hamsters. *, $P < 0.05$ vs. controls (Cont.).

(Fig. 5) and a lighter staining in the VMH/VLH, especially in diabetic animals. There was no difference in the effectiveness of these two treatments.

Exp 3: AP lesions

Histological analysis showed that 22 out of 26 of the AP lesioned animals received complete destruction of the AP with varying amounts of damage to the underlying NTS (Fig. 6). The presence or absence of this damage to the NTS did not affect either estrous behavior or neural ERIR. The lesioned animals seemed to be quite healthy, with no observable signs of illness. The final group size was 11 for each lesioned group.

In neurologically intact animals, treatment with metabolic inhibitors for 48 h caused a significant suppression in the duration of lordosis ($P < 0.05$), a decrease in the number of ERIR cells in the VMH/VLH ($P < 0.001$) and an increase in ERIR cells in the mPOA ($P < 0.05$) (Fig. 7). AP lesions abolished the suppressive effect of metabolic inhibitors on lordosis and VMH/VLH ERIR but did not alter their effect on ERIR in the mPOA (Fig. 7).

Exp 4: vagotomy

Twenty out of 27 of the vagotomized animals showed complete absence of fluorogold in the dorsal motor nucleus of the vagus, and successful fluorogold injection was confirmed by the presence of fluorescence within the AP. The final group size was 10 for each vagotomized group.

In sham-vagotomized animals, treatment with metabolic inhibitors for 48 h caused a significant suppression in the duration of lordosis ($P < 0.01$), a decrease of the number of ERIR cells in the VMH/VLH ($P < 0.05$) and an increase in ERIR cells in the mPOA ($P < 0.01$) (Fig. 8). Vagotomy abolished the effects of metabolic inhibitors on the number of ERIR cells in the mPOA, but it did not prevent the attenuation of lordosis duration ($P < 0.05$) or decrease in the number of ERIR cells in the VMH/VLH ($P < 0.05$) (Fig. 8).

Discussion

Restriction of metabolic fuel availability by food deprivation, treatment with metabolic inhibitors, or streptozo-

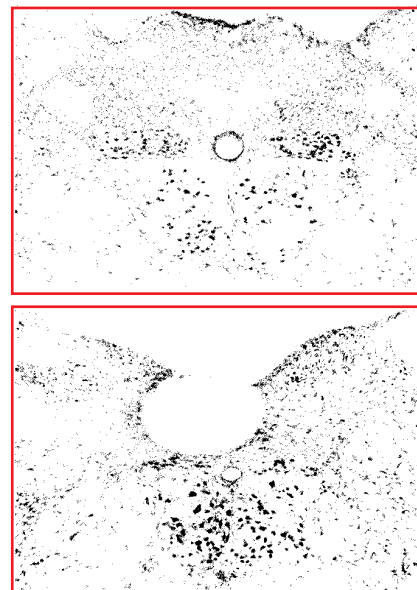


FIG. 6. Photomicrographs of sections stained with cresyl violet illustrating an intact AP (top) and an AP lesion with some ablation of the adjacent NTS (bottom).

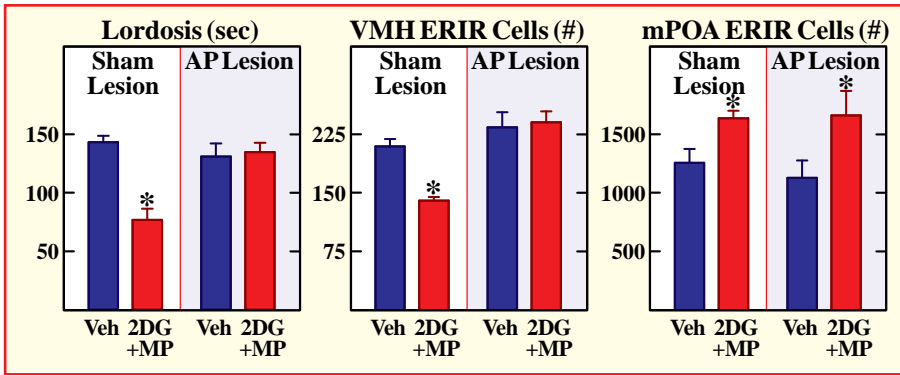


FIG. 7. Effects of treatment with vehicle or 2DG (750 mg/kg) and MP (25 mg/kg) once every 6 h for 48 h on lordosis duration, ERIR cell number in the VMH/VLH, and ERIR cell number in the mPOA of ovariectomized hamsters that were either sham-operated or given lesions of the AP. *, $P < 0.05$ vs. controls.

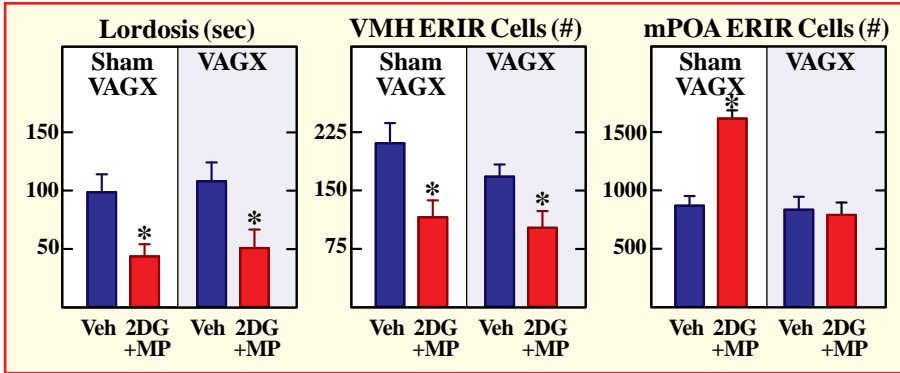


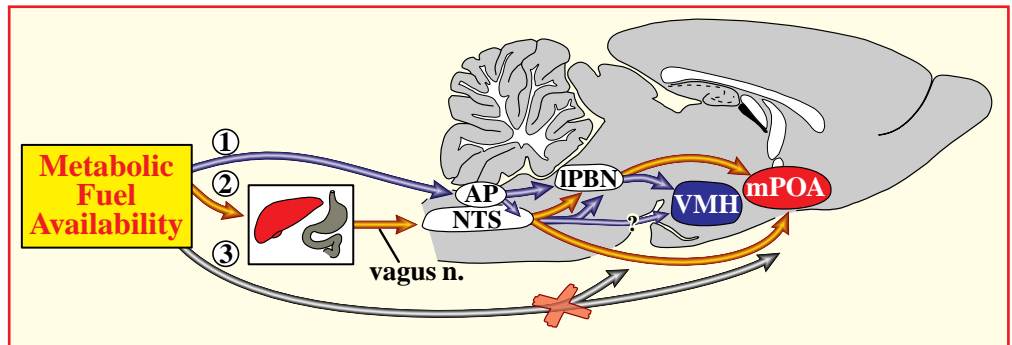
FIG. 8. Effects of treatment with vehicle or 2DG (750 mg/kg) and MP (25 mg/kg) once every 6 h for 48 h on lordosis duration, ERIR cell number in the VMH/VLH, and ERIR cell number in the mPOA of ovariectomized hamsters that were either sham-operated or vagotomized (VAGX). *, $P < 0.05$ vs. controls.

tocin-induced diabetes diminished the number of detectable ERIR cells in the VMH/VLH. These findings support the hypothesis that the reduced behavioral responsiveness to exogenous steroids in fuel-deprived, ovariectomized hamsters is at least in part due to a decrease in estradiol binding in the VMH/VLH. The VMH is probably a pivotal locus for the facilitation of lordosis by estradiol and progesterone (19, 37, 38). In female hamsters, bilateral lesions in the VMH eliminate steroid-induced lordosis (21), whereas implants of estradiol restricted to the VMH are sufficient to facilitate female sexual behavior (19, 37, 38). Moreover, an antiestrogen applied directly to the VMH can block the expression of lordosis in rats (39), indicating that estradiol specifically in the VMH is necessary for lordosis.

It is probably noteworthy that the VMN proper and the estrogen-receptor-rich region extending immediately lateral to it (VLH) responded identically to all of the manipulations of metabolic fuel availability that were used in this work. Thus, it is conceivable that the estrogen-binding neurons in the ventral hypothalamus that participate in the expression of estrous behavior include both the VMN proper and the area extending laterally from the nucleus.

FIG. 9. Working hypothesis as to how metabolic fuel information might be transmitted to ERIR neurons in hamster forebrain. ① Metabolic fuel information reaches the VMH via a pathway (blue arrows) that includes the AP and perhaps the lateral parabrachial nucleus (PBN). It is possible that metabolic fuel availability is detected in the AP. ② Metabolic fuel information reaches the mPOA via a pathway (red arrows) that includes the vagus nerves and possibly the NTS and PBN. It is possible that metabolic fuel availability is detected in the viscera. ③ These experiments provide no evidence that ERIR cells in the VMH or mPOA detect metabolic fuel availability directly (gray arrows).

Our findings are consistent with the notion that suppression of estrous behavior requires both glucoprivation and lipoprivation (8), as treatment with both 2DG and MP was required to diminish detectable ERIR in the VMH/VLH. In contrast, glucoprivation alone is sufficient to suppress estrous cycles and suppress *Fos* expression in forebrain GnRH cells (10, 12), whereas lipoprivation alone can cause a hibernation-like torpor in gonadally intact female hamsters (10). These results suggest that, unlike metabolic



control of estrous behavior, estrous cycles and GnRH release are sensitive to changes in glucose availability, regardless of the availability of other metabolic substrates.

The effects of food deprivation and glucoprivation + lipoprivation on neural ERIR are site-specific, because fuel restriction decreased ERIR in the VMH/VLH, increased ERIR in the mPOA, and had no effect in the NTS. The significance of the deprivation-induced increase in ERIR in the mPOA is not clear. The mPOA plays a role in a number of estrogen-sensitive functions, including estrous behavior (20, 22), maternal behavior (40, 41), and gonadotropin secretion (42), all of which are affected by food deprivation (3-5, 8, 11, 43). It is not immediately obvious how increased mPOA ERIR might be involved in the effects of food deprivation on these endpoints.

Our findings in diabetic female hamsters are consistent with the idea that a decrease in neuronal estrogen binding may decrease lordosis in response to steroid hormones in diabetic female rats (17). Our work, using immunocytochemistry, with its much higher anatomical resolution, confirms that insulin withdrawal decreases ERIR in the VMH/VLH in hamsters. As expected, estrous behavior was suppressed in diabetic hamsters, and it was restored by insulin replacement therapy. The fact that both glucoprivation and lipoprivation are required to decrease estrous behavior and ERIR in nondiabetic animals may mean that insulin deficiency impairs both glucose and fatty acid utilization in hamsters. The finding that feeding a high-fat diet does not ameliorate effects of diabetes on estrous behavior in hamsters (Li and Wade, unpublished results) as it does in rats (16) is consistent with this possibility.

AP lesions and vagotomy had different effects on lordosis and ERIR level in response to metabolic fuel restriction. AP lesions abolished the effects of metabolic inhibitors on lordosis and the number of VMH/VLH ERIR cells but did not affect mPOA ERIR. On the other hand, vagotomy abolished the effects of metabolic inhibitors on mPOA ERIR but did not affect either lordosis or VMH/VLH ERIR. Thus, expression of lordosis is consistently associated with ERIR levels in the VMH/VLH but not in the mPOA. The fact that we were able to dissociate the effects of metabolic fuel manipulations on estrous behavior and the number of detectable ERIR neurons in the mPOA suggests that metabolic fuel availability does not affect lordosis via mPOA estrogen receptors. These findings also seem to rule out the possibility that food deprivation or metabolic inhibitors affect estrous behavior and neural estrogen binding because of stress, a general malaise, or some nonspecific toxicity. It is unlikely that additional neurological damage (AP lesions or vagotomy) would make these manipulations any less stressful or toxic.

Our findings suggest that estrogen-binding neurons in the VMH/VLH and mPOA do not detect metabolic fuel availability directly, because metabolic effects on ERIR in both regions were abolished by specific damage elsewhere in the nervous system. The neural pathway that conveys metabolic information to the VMH/VLH includes at least the AP, but not the vagus nerves. Conversely, the vagus nerves, rather than the AP, are required for mPOA to receive information concerning fuel availability. How, then, do the AP and vagus nerves transmit metabolic signals to their target areas? As mentioned previously, the AP lies outside the blood-brain barrier and is able to detect circulating substances, and it may sense metabolic status. In rats, a major efferent pathway of the AP/NTS extends to the lateral parabrachial nucleus in the dorsal pons; from there the pathway extends monosynaptically to the thalamus, hypothalamus and limbic forebrain (44). These results suggest that the VMH could receive information about metabolic fuel availability from the AP via the lateral parabrachial nucleus. To determine the role of the lateral parabrachial nucleus in mediating metabolic controls of estrous behavior and neural estrogen binding, it would be worthwhile to investigate the effects of lesions of the lateral parabrachial nucleus on estrous behavior and neural ERIR in response to metabolic inhibitors.

The NTS is the major termination zone of visceral afferents in the vagus nerve. The fact that vagotomy, but not AP lesions abolished the effects of metabolic inhibitors on mPOA ERIR suggests that mPOA receives information about metabolic fuel availability via the vagus without the participation of the AP. A direct projection from neurons in the mid-dorsal portion of the NTS, an area that was not destroyed by the AP lesions in this study, to the mPOA and several other forebrain areas provides a pathway that may transmit metabolic fuel information to the mPOA (30). It is conceivable that inadvertent damage to the NTS could account for some of the effects of the AP lesions. However, the fact that the effects of vagotomy were not mimicked by AP lesions would seem to argue against this possibility.

Although the results presented here suggest potential neural pathways by which the VMH/VLH and mPOA may receive information about metabolic fuel availability (Fig. 9), they do not address the question of where the metabolic detectors are; nor do they provide any information about the transduction processes that convert metabolic cues into changes in the number of detectable ERIR neurons.

Several conclusions follow from this work. First, in addition to any steroid-independent mechanisms (11), food availability can affect reproductive function by altering neural responsiveness to ovarian steroids, possibly by changing neuronal steroid receptor levels. Fuel restriction can attenuate some responses to steroid treatment (*e.g.*, estrous

behavior), whereas the mPOA ERIR findings are consistent with the possibility that some physiological or behavioral responses to estradiol may be enhanced during food deprivation (45). Second, the various aspects of nutritional infertility appear to be at least partially independent of one another, responding to different metabolic cues (8, 10) and using different neural pathways. Finally, this and related work (46) illustrate the validity of the notion (2, 3) that the concepts and techniques that have been used to study physiological controls of feeding behavior (*e.g.*, 23, 24) can be applied with great utility to the study of nutritional infertility as well as to other physiological and behavioral responses to food availability.

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