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Reduction of Glucose Availability Suppresses Pulsatile Luteinizing Hormone Release in Female and Male Rats*

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ABSTRACT

Glucose availability controls reproductive activity through modulation of LH secretion. The aim of the present study was to determine whether the glucoprivic suppression is potentiated by gonadal steroids and if glucoprivic suppression of pulsatile LH release is sexually differentiated. Pulsatile LH secretion was examined in rats after peripheral (jugular) administration of the competitive inhibitor of glycolysis, 2-deoxyglucose (2DG). Fourteen days after gonadectomy, blood samples were collected every 6 min for 3 h. One hour after the onset of sampling, 2DG was administered peripherally (200, 400, or 800 mg/kg BW, iv), and food intake was determined after 2DG injection in gonadectomized males and females in the presence or absence of sex steroids (testosterone or estradiol). To test the ability of the pituitary to produce LH under glucoprivic conditions, LHRH was injected every 30 min for 2.5 h in ovariectomized (OVX) rats 30 min after treatment with 400 mg/kg 2DG. At all peripheral doses of 2DG in females and at the middle and high doses of 2DG in males, mean plasma LH and LH pulse frequency decreased ($P < 0.05$) in the presence of steroids. However, in the absence of sex steroids, the lowest dose in females and the middle dose in males were not effective. Pituitary function appeared normal, because increases in mean plasma LH in response to the exogenous LHRH occurred in OVX rats treated with the middle dose of 2DG. Food intake significantly ($P < 0.05$) increased after 2DG injection in all groups except estrogen-treated OVX females at the low and high doses of 2DG. These findings suggest that glucoprivic suppression of LH pulses is potentiated by gonadal steroids in both sexes. Moreover, the hypothalamo-hypophyseal axis of the female rat seems to be more sensitive to the decreased glucose availability induced by 2DG than that of the male. (*Endocrinology* **137:** 1166–1170, 1996)

FOOD DEPRIVATION interrupts steroid-induced estrous behavior in ovariectomized (OVX) Syrian hamsters (1) and suppresses LH secretion in a variety of species, including humans (2), monkeys (3), sheep (4), and rats (5, 6). During the fasted condition, glucose availability may play an important role in regulating gonadal activity through the modulation of LH secretion (7, 8). Infusion of glucose facilitated pulsatile LH secretion within hours in nutritionally growth-delayed prepubertal lambs (9) and prevented the suppression of pulsatile LH release caused by insulin-induced hypoglycemia in ewes (10). In Syrian hamsters, glycoprivation induced by 2-deoxyglucose (2DG), a competitive inhibitor of glucose utilization, interrupted estrous cyclicity, but lipoprivation induced by methyl palmoxirate, an inhibitor of fatty acid oxidation did not (11). Interestingly, steroid-induced estrous behavior in OVX Syrian hamsters is suppressed only during combined glucoprivation and lipoprivation.

Our previous studies revealed that steroids can modify the degree of fasting-induced suppression of LH secretion (12, 13) by a feedback action of estrogen at the paraventricular nucleus or A2 (14). In the present study, we assessed whether gonadal steroids can modulate glucoprivation-induced suppression of pulsatile LH secretion. To determine whether glucoprivic suppression of pulsatile LH secretion is sexually differentiated, we examined the effect of peripheral 2DG injection on pulsatile LH secretion in gonadectomized or gonadal steroid-treated [testosterone (T) or 17β-estradiol (E_2)] gonadectomized male and female rats.

Materials and Methods

Animals

Adult female and male Wistar-Imamichi strain rats, weighing 220–270 g, were used. They were individually housed under a controlled environment (14 h of light, 10 h of darkness; lights on at 0500 h; 24 ± 2 C) and provided with food (Labo-MR-stock, Nihon Nosan Kogyo Co., Yokohama, Japan) and water *ad libitum*. The food was powdered on the day of sampling to facilitate measurement of its intake.

Females that showed at least two consecutive 4-day estrous cycles or males were gonadectomized to eliminate endogenous sex steroids. At gonadectomy, some animals of each sex were given chronic steroid treatment. Females received a sc implant consisting of silicone tubing (id, 1.5 mm; od, 3.0 mm; length, 25 mm; Kaneka Medix, Osaka, Japan) containing E_2 (Sigma Chemical Co., St. Louis, MO) dissolved in peanut oil at 20 μg/ml; males were implanted with tubing (id, 2.64 mm; od, 4.88 mm; length, 12.5 mm; Dow Corning, Midland, MI) containing crystalline T (Sigma).

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Experimental protocols

Fourteen days after gonadectomy, blood samples (100 μl) were collected every 6 min for 3 h through an indwelling atrial cannula (silicone tubing; id, 0.5 mm; od, 1 mm; Shin-Etsu Polymer Co., Tokyo, Japan) that had been inserted on the day before the sampling. An equivalent volume of rat red blood cells from donor animals was suspended in saline and replaced in experimental animals at each sampling. One hour after the start of sampling, 2DG (Sigma grade III) dissolved in saline (200, 400, or 800 mg/kg BW) was administered through the atrial cannula. These doses of 2DG were chosen based upon data from the lamb in which a dose of 480 mg/kg was effective peripherally in reducing LH pulse frequency (15). To assess pituitary responsiveness, five OVX animals treated with 400 mg/kg 2DG were administered LHRH (Peptide Institute, Osaka, Japan; 100 ng/kg BW dissolved in saline) every 30 min for 2.5 h through the atrial cannula starting 30 min after 2DG administration. This dose of LHRH was chosen because it produced a LH pulse of normal size (16). Plasma was separated by immediate centrifugation and stored at −20 C until assayed for LH. Food intake was measured during the 2-h sampling period after 2DG injection.

Hormone assay

Plasma LH concentrations were determined by a double antibody RIA with a rat LH RIA kit provided by the National Hormone and Pituitary Program (Baltimore, MD) and were expressed in terms of the reference preparation NIDDK rat LH RP-3. The least detectable level of LH was 0.16 ng/ml for 50-μl plasma samples, and the intra- and interassay coefficients of variation were 4.10% and 9.54% at 0.7 ng/ml, respectively.

Statistical analysis

LH pulses were identified by the Pulsar computer program (17) using the parameters previously described (18). The mean LH concentration and the frequency and amplitude of LH pulses both pre- and post-2DG treatment were calculated for each individual and then for each group. Percent changes in mean plasma LH concentration, LH pulse frequency and amplitude after 2DG injection were determined by calculating the ratio of the difference in plasma LH concentrations between the pre- and post-2DG injection periods to that during the pre-2DG injection period in individual animals. Statistical differences in the percent change in mean LH concentration, the frequency and amplitude of LH pulses, and food intake between groups were determined by the Mann-Whitney U test. Statistical differences in mean LH concentrations were determined by the Wilcoxon signed rank test.

Results

Plasma LH concentrations

Representative profiles of plasma LH levels in individual females and males injected with various doses of 2DG are shown in Fig. 1. Percent changes in mean LH concentration and the frequency and amplitude of LH pulses after 2DG treatment are shown in Fig. 2. Regular LH pulses were observed in saline-treated rats throughout 3-h sampling period and during the preinjection period in 2DG-treated rats. In the absence of steroids, the mean LH concentration and frequency of LH pulses were significantly ($P < 0.05$) decreased by the middle (400 mg/kg BW) and high (800 mg/kg BW) doses of 2DG in OVX females and by only the high dose in castrated males. However, in the presence of steroids, the mean plasma LH concentration in both gonadectomized females and males was significantly ($P < 0.05$) decreased after all doses of 2DG compared to that in saline-injected controls. Significant ($P < 0.05$) decreases in the frequency of LH pulses occurred in females after all doses of 2DG and in males after the middle and high doses of 2DG. There was a significant ($P < 0.05$) decrease in the amplitude of LH pulses in E_2-treated OVX females after the middle and high doses of 2DG compared to that in saline-injected controls.

Mean LH concentrations increased in response to each

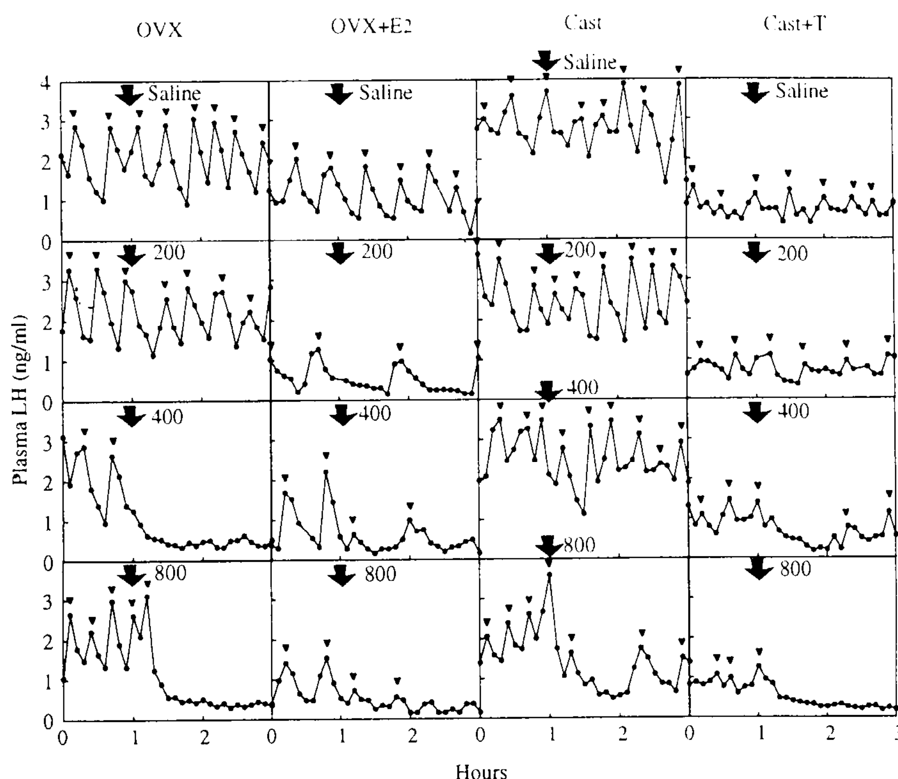

FIG. 1. Representative profiles of pulsatile LH secretion in gonadectomized or steroid-treated gonadectomized female and male rats before and after iv injection of saline or 2DG (200, 400, or 800 mg/kg BW). *Arrows* represent the timing of injections. *Arrowheads* indicate the peaks of LH pulses identified by the Pulsar computer program.

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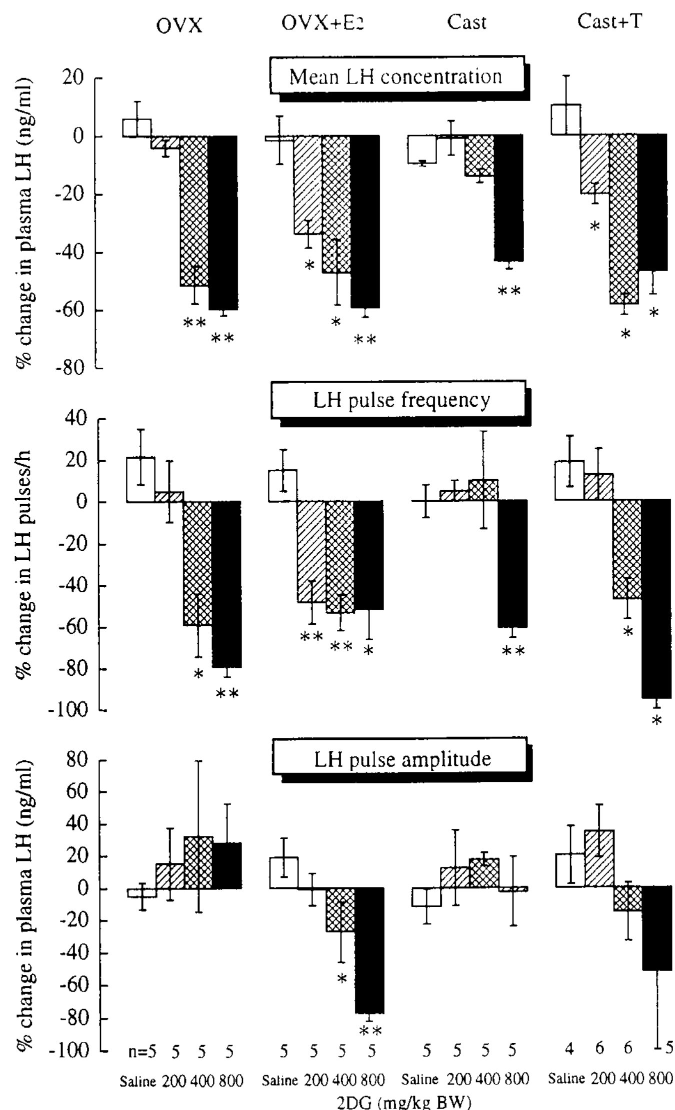

FIG. 2. Percent changes in mean plasma LH concentrations, LH pulse frequency, and LH pulse amplitude induced by 2DG in gonadectomized or steroid-treated gonadectomized female and male rats. Values are the mean ± SEM. *, $P < 0.05$; **, $P < 0.01$ (*vs.* saline-treated controls).

LHRH administration at 30-min intervals in gonadectomized females after treatment with the middle dose of 2DG (Fig. 3, a and b). In rats not receiving the LHRH injection (Fig. 3, c and d), 2DG significantly ($P < 0.01$) decreased mean LH concentrations.

Food intake

In the absence of steroids, food intake significantly ($P < 0.05$) increased in OVX females after the low and middle doses of 2DG compared to that in the saline-injected controls. In E_2-treated females, the increase in food intake was noted at the middle dose of 2DG (Fig. 4). In both castrated and T-treated castrated males, all doses of 2DG significantly ($P < 0.05$) increased food intake.

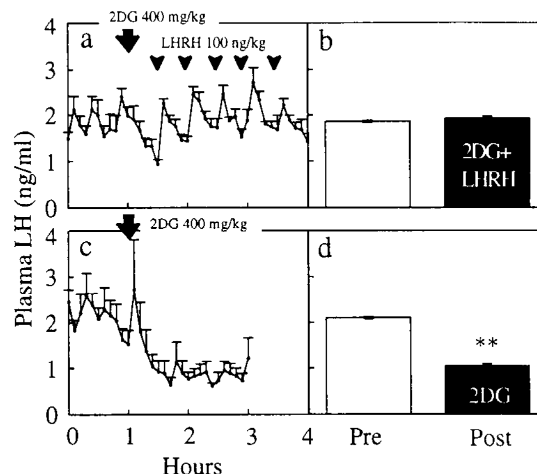

FIG. 3. Changes in mean plasma LH concentrations in OVX rats treated with 2DG and injected with LHRH every 30 min after 2DG treatment (a) or not treated with LHRH (c). *Left panels* present mean pre- and posttreatment concentrations of LH in OVX rats treated with (b) or not treated (d) with LHRH. *Arrows* and *arrowheads* indicate the 2DG and LHRH injections, respectively. Values are the mean ± SEM (n = 5). **, $P < 0.01$ *vs.* pretreated controls.

Behavior

Most of rats became sedated after the high dose of 2DG in both OVX and E_2-treated OVX groups. This was not the case with males treated with any doses of 2DG. 2DG did not have an apparent behavioral effect in castrated or T-treated males.

Discussion

The present study reveals that in female and male rats, glucose availability is important in the control of gonadotropin secretion, because 2DG treatment suppressed pulsatile LH secretion in a dose-dependent manner. 2DG did not render the pituitary nonfunctional, because it responded to exogenous LHRH. The dosage of LHRH used in this study (100 ng/kg BW) induced physiological amounts of LH release in the previous study (16). In the lamb, LHRH (5 ng/kg BW, iv) or *N*-methyl-D,L-asparate (5 mg/kg BW, iv), which are known to activate LHRH neurons (19), induced LH release after peripheral 2DG treatment at a dose (480 mg/kg BW) known to suppress LH secretion (15). Taken collectively, these findings suggest that the suppressive effect of 2DG on LH pulses is probably mediated by suppression of LHRH release. This conclusion must remain tentative until detailed studies of the effects of 2DG-induced glucoprivation are conducted on LHRH secretion (pituitary portal circulation) or on pituitary sensitivity to LHRH (dose response).

The present study was not intended to resolve whether 2DG administered peripherally acts at the central nervous system to suppress pulsatile LH release. It could act indirectly. Peripheral injection of 2DG induces hyperglycemia in response to increases in plasma epinephrine levels (20) and glucagon release (21) and to inhibition of insulin secretion (22). These physiological counterregulatory responses to glucoprivation could, in turn, be involved in the inhibition of LH release. For example, LH secretion was suppressed in streptozotocin-treated diabetic rats, which were hyperglycemic

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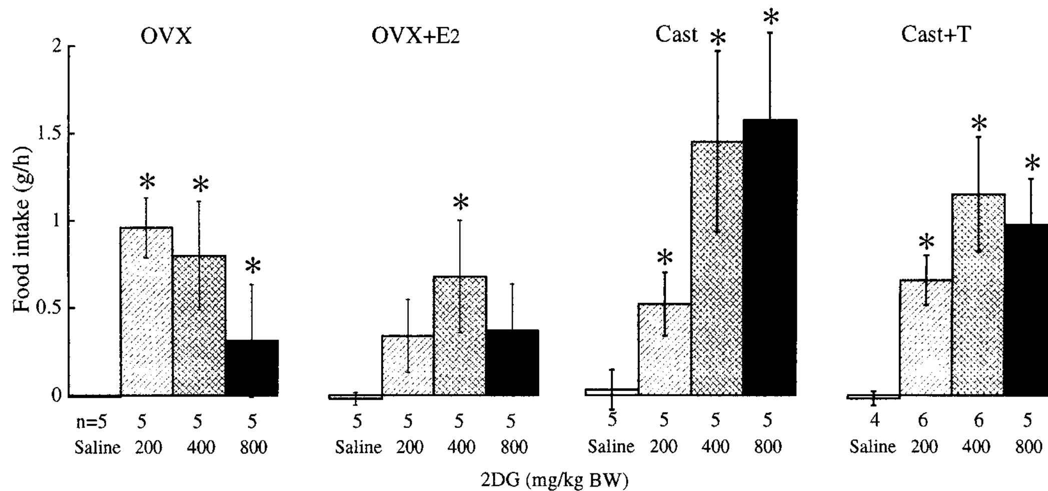

FIG. 4. Food intake after the injection of 2DG in gonadectomized female and males rats with or without steroid treatments. Values are the mean ± SEM. *, $P < 0.05$ *vs.* saline-treated controls.

due to decreased insulin release (23). On the other hand, there is increasing evidence that central sensors in the caudal brain stem mediate the regulation of LH secretion by glucose availability. In this regard, 2DG-induced inhibition of estrous cycles in the Syrian hamster was abolished by lesion of the area postrema (24), and intracerebroventricular administration of 2DG was effective in suppressing LH pulse frequency in the lamb (15). We recently determined that administration of 2DG exclusively into the fourth ventricle of both castrated and T-implanted castrated male rats suppresses pulsatile LH release at a much smaller dose (40 mg/kg BW) than those used in the present study (200, 400, and 800 mg/kg) (25).

There was a sex difference in the inhibitory effect of 2DG on LH release in the absence of gonadal steroids. When females and males were treated with 400 mg/kg 2DG, suppression of pulsatile LH release was greater in females. A tendency for a similar difference was also found in the presence of gonadal steroids when both sexes were treated with 200 mg/kg 2DG. Perhaps, the LHRH mechanism of females may be more sensitive to 2DG-induced glucoprivation than that of males. In rodents, nutritionally growth-restricted males become sexually mature, but females do not do so until nutrient intake increases (26). This may make some sense to prevent pregnancy and lactation, which are very expensive energetically; to avoid the risk of conception, females might reduce LH secretion in response to low available energy more readily than males.

We found that gonadal steroids enhanced 2DG-induced suppression of LH secretion, because a low dose (200 mg/kg BW) of 2DG was effective in the presence of steroids, but not in their absence. The mechanism by which estrogen enhances the suppressive effect of glucoprivation on LH pulses is unknown. In our previous study, estrogen was required to suppress pulsatile LH secretion in rats after a 48-h fast (12, 13). The activation of CRH neurons is involved in the fasting-induced suppression of LH release in E_2-treated OVX rats because intracerebroventricular injection of α-helical CRH-(9–41), a CRH antagonist, blocked the inhibitory effect of fasting on LH release (27). Provided that a reduction in glucose availability was playing a role in the suppression of LH release after a 48-h fast, we hypothesize that moderate glucoprivation (low dose, 200 mg/kg BW 2DG) in this study stimulated CRH neurons to suppress pulsatile LH release with the aid of gonadal steroids. However, considering that in both sexes some dose of 2DG was able to suppress LH pulses independent of estrogen, it is possible that unlike fasting, glucoprivic suppression of LH release involves a mechanism other than CRH neurons.

Glucoprivation induced by 2DG is known to induce feeding behavior in satiated rats (28). In the present study, peripheral injection of 2DG increased food intake in both females and males at all doses examined compared to that in saline-injected controls. The low dose of 2DG was more effective in increasing food intake in gonadectomized rats, although it failed to suppress LH pulses. Perhaps, there is a difference in the sensitivity to glucoprivation by 2DG between the mechanisms regulating food intake and pulsatile LH release. However, food intake was less at the high dose compared to that at the low or middle dose in OVX rats and to that at the middle dose in E_2-treated OVX rats in the present study. This may be due to the sedative effect of 2DG at the high dose. Therefore, we cannot rule out the possibility that a malfunction of the central nervous system may be partly involved in the suppression of LH secretion after the injection of 2DG at the high dose.

In conclusion, our present results suggest that suppression of pulsatile LH secretion by the 2DG-induced decrease in glucose availability can be potentiated by gonadal steroids in both males and females. Females may be more sensitive than males to glucoprivation by 2DG, resulting in the suppression of LH release.

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