A Critical Role for the Evolutionarily Conserved Gonadotropin-Releasing Hormone II: Mediation of Energy Status and Female Sexual Behavior

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GnRH is an evolutionarily conserved neuropeptide, of which there are multiple structural variants; the function of the most widespread variant, GnRH-II, remains undefined. GnRH-II may affect reproductive behavior; GnRH-II administration to female musk shrews reinstates mating behavior previously inhibited by food restriction. To determine whether this action of GnRH-II is universal, we conducted the following studies in mice. Ovariectomized mice were primed with estradiol benzoate and progesterone once a week and tested for sexual behavior. Females showing a lordosis quotient (LQ) of 50 or higher on the fourth trial underwent food deprivation (FD) for either 24 or 48 h before an additional behavior test. FD for 48 h significantly reduced LQ compared with ad libitum-fed females. Next, females were FD for 48 h or maintained on ad libitum feeding and retested for sexual behavior after an intracerebroventricular infusion of either GnRH-I, GnRH-II, or saline. GnRH-II, but not GnRH-I, significantly increased LQ in FD females compared with FD females treated with saline. Lordosis was unaffected by GnRH-II in females maintained on ad libitum feeding. To assess whether the GnRH-I receptor mediates GnRH-II's behavioral effects, underfed females were pretreated with the type 1 GnRH receptor antagonist Antide and retested for sexual behavior. Antide pretreatment did not prevent GnRH-II from promoting mating behavior, suggesting that GnRH-II's behavioral actions are mediated through the type 2 GnRH receptor. We speculate that GnRH-II acts via its own receptor as a regulatory signal in mammals to ensure that reproduction is synchronized with energetically favorable conditions. (Endocrinology 145: 3639–3646, 2004)

Although GnRH II can promote LH secretion in mammals, it does so with a much lower potency than GnRH I (~2% as effective) (21, 22). Furthermore, in sheep and primates, GnRH II stimulation of pituitary gonadotropins is completely blocked with administration of a type 1 GnRH receptor antagonist (23, 24), suggesting that GnRH II promotes LH release by activating type 1 GnRH receptors in the pituitary. In mammals, the majority of GnRH II cell bodies reside in the midbrain, with few cells present in hypothalamic and extrahypothalamic regions (7–9). In addition, only a minority of the GnRH II neurons in the mammalian brain project to forebrain regions that regulate gonadotropin secretion; in the musk shrew, the majority of GnRH II-containing terminals are present in the medial habenula (25). Finally, in amphibian neurons, GnRH II plays a neuroregulatory role by modulating M currents through K+ and Ca2+ channels (26, 27). Collectively, these findings suggest that the primary role of GnRH II is not to stimulate gonadotropin hormones but rather to act as a neurotransmitter (28).

Temple et al. (20) proposed that GnRH II, acting as a neurotransmitter, regulates reproduction according to a female’s energetic status. In female mammals, reproduction is an energetically costly process; in mice, rats, and hamsters, low food availability delays the onset of puberty (29, 30), suppresses ovulation and estrous cycles in adults (31–37) and reduces rates of successful parturition (35, 36, 38). Furthermore, in hamsters and rats, low energy availability dramatically impairs female mating behaviors (20, 39–44), even in steroid-primed, ovariectomized adults (40, 41, 43, 44). Similarly, female musk shrews that are food restricted for 48 h...
exhibit a significant decline in mating behaviors; interestingly, central administration of GnRH II, but not GnRH I rapidly reverses the inhibitory effects of food restriction on female musk shrew sexual behavior (20). In contrast, GnRH II does not further increase reproductive behavior in ad libitum-fed shrews (45), implying that this peptide’s function in reproduction is likely permissive and not merely stimulatory. In addition, GnRH II infusion to ad libitum-fed or food-restricted females produces a short-term decrease in food intake (46). Therefore, we propose that GnRH II acts as a neurotransmitter to reflect the energetic state of a female, promoting reproductive behavior and depressing feeding when sufficient energy is available; under this model, when females are not energetically challenged, more GnRH II is released, thereby decreasing feeding and concurrently permitting mating.

To ask if GnRH II’s novel role of permitting female reproductive behavior generalized to other mammals, we conducted the present studies in mice. Unlike musk shrews, adult female mice exhibit persistent estrous cycles in gonadal hormone production and sexual behavior. Although there is abundant evidence indicating that food deprivation (FD) can inhibit estrous cycles and ovulation in mice (35–37, 47), surprisingly, no studies have directly addressed the effects of low food availability on female sexual behavior in these rodents. First, we assessed the impact of varying durations of short-term FD on the mating behavior of female mice and evaluated the time-course of reinstatement of behavior after brief re-access to food. Next, we compared the effects of central GnRH I and II administration on sexual behavior in both ad libitum-fed and underfed female mice. Lastly, we determined whether, similar to its stimulatory effects on gonadotropin release in mammals, GnRH II’s permissive actions on female mammalian reproductive behavior are mediated by the type 1 GnRH receptor.

Materials and Methods

Subjects

All studies used adult (2–3 months of age), initially sexually naive female C57BL/6J mice purchased from The Jackson Laboratory (Bar Harbor, ME). Mice were group housed with food (Harlan Diet 8604; Harlan Teklad, Madison, WI) and water available ad libitum (unless noted differently in specific experiments) until several days before trial 1 of experiment 1, at which time they were housed individually. The room was maintained on a 12-h light, 12-h dark photoperiod (lights off at 1200 h) at a temperature of 23 ± 2 C. All procedures were approved by the Animal Care and Use Committee of the University of Virginia.

Ovariectomies and stereotaxic implantation of cannula

Ovariectomies were conducted under general anesthesia (100 mg/kg ketamine and 10 mg/kg xylazine injected ip) 10–20 d before trial 1. Females received an intracerebroventricular (icv) cannula after trial 6 (see Experiment 2). To implant cannulas, females were anesthetized (100 mg/kg ketamine and 10 mg/kg xylazine injected ip) and fit into a mouse stereotaxic apparatus (Kopf Instruments, Tujunga, CA). A mid-line incision was made along the top of the head exposing bregma; guide cannulas (26 gauge; Plastics One, Roanoke, VA) containing an internal dummy cannula were centered on bregma and moved −0.2 mm rostral-caudal and −1.0 mm medial lateral. A hole was then drilled in the skull and the cannula lowered to a depth of 2.8 mm, aimed at the lateral ventricle. The cannula was fixed to the skull with glue and dental acrylic, and the animal was kept warm until it regained consciousness.

Peptide infusions

For icv infusions, females were briefly anesthetized with isoflurane inhalant and infused with 10 µl of either vehicle (0.09% saline), GnRH I (1 µg; Sigma, St. Louis, MO), or GnRH II (1 µg; Bachem, Torrance, CA). The amounts of GnRH used were based on previously published reports showing that these doses are sufficient to stimulate ovulation and/or female mating behavior (20, 45). All infusions were made using an internal cannula (23 gauge) with a 0.1-mm projection attached to a syringe and delivered slowly by hand over the course of 30–40 sec. The internal cannula was left in place for 30–45 sec after infusion to prevent backflow, after which the dummy cannula was replaced and the female returned to her cage. Females aroused from anesthesia within 1 min after the infusion. At the end of the study, cannula placement was confirmed; females were killed by lethal injection (sodium pentobarbital) and 0.01 ml India ink injected into the cannula. The brains were removed and sectioned on a cryostat. Cannulas were considered correctly placed if the ventricles were filled with ink.

Sexual behavior tests

Forty-eight hours before each mating test, females received estradiol benzoate (EB; 0.5 µg/0.5 ml sesame oil, sc) followed by a progesterone injection (P; 500 µg/0.3 ml sesame oil, sc) 3–5 h before testing. To test female sexual behavior, mice were paired with stud males (of either C57BL/6J or DBA strains). Stud males were acclimated to the testing box 30–60 min before the introduction of the female; each test lasted 20 min, or until the male had either mounted the female 20 times or mounted fewer times and ejaculated. The number of times the male mounted and the number of times the female displayed lordosis were recorded. A lordosis quotient (LQ) for each test was determined by dividing the number of times a female displayed lordosis by the total number of mounts received, and multiplying this value by 100.

Antide dose-response studies

To determine a dose of Antide that effectively blocks the type 1 GnRH receptor (for experiment 3), two preliminary studies were conducted. In the first, plasma LH concentrations were measured in ovariectomized, cannulated females 3 h after icv administration of Antide (0.1 µg, 1.0 µg) or saline (6–8 animals per group; animals ovariectomized 4–6 wk beforehand). In the second study, plasma LH was measured in additional ovariectomized, cannulated females that were primed beforehand with EB and P (in a time-course identical with females tested for sex behavior). For this part, Antide (1 µg; n = 10) or saline (n = 10) was given icv several hours before an infusion of GnRH I (1 µg) and blood collected 30 min later. In all cases, blood was collected via retro-orbital bleeding of mice under light anesthesia (isoflurane). The LH RIA was conducted by the University of Virginia Core Ligand and Assay Laboratory; we employed a modified supersensitive two-site sandwich immunoassay using monoclonal antibodies MABI (no. 58187) against bovine LH and TMA (no. 5303; Medix Biochemicals, Kauniainen, Finland) against human LH. This LH assay has been previously validated for mouse (48). The assay has a sensitivity of 7 pg/tube, and the intraassay coefficient of variation of the quality controls ranged from 0.7–9.7%.

Experiment 1: establishment of paradigm for assessing effects of FD sex behavior

Adult females (n = 36) were ovariectomized several weeks before the start of the study. Each female received four sexual behavior tests, one test every 5–7 d. Females showing a LQ of 50 or higher on the fourth behavior trial underwent FD for either 24 or 48 h, or remained on ad libitum feeding before an additional behavior test (trial 5; n = 12 per group; groups matched for mean body weight and LQ). After the fifth behavior trial, females were returned to ad libitum feeding and tested again 7 d later (trial 6). An additional 40 ovariectomized females that displayed a LQ of at least 50 on the fourth behavior trial were randomly divided into four groups matched for mean body weight and LQ (n = 10 per group): one group remained on ad libitum feeding; the other three groups were food deprived for 48 h and then returned to ad libitum feeding for either 0, 3, or 8 h before the next behavior test (trial 5). After
trial 5, all animals were returned to ad libitum feeding and retested 1 wk later (trial 6). For all animals, body weights were recorded on trials 4–6.

**Experiment 2: effects of gonadotropin-releasing hormones on female sex behavior**

Female mice from experiment 1 received one additional sex behavior test while on ad libitum feeding and the following day were surgically implanted with a cannula aimed at the lateral ventricle. Five days later, the females were randomly divided into six groups matched for mean body weight and LQ (n = 10–12 animals per group). Females in half the groups remained on ad libitum feeding, whereas the other mice were food deprived for 48 h before the next behavior test (Table 1). On the day of testing, all animals received an icv infusion of either GnRH II (1 μg), GnRH I (1 μg), or vehicle (saline) 20 min before the beginning of the test (all animals were primed with EB and P as described previously). After testing, females were returned to ad libitum feeding. Body weights were recorded on each of the testing days.

**Experiment 3: effect of type 1 GnRH receptor blockade on sexual behavior**

Seven to 10 d after the conclusion of experiment 2, cannulated females (n = 32) were randomly divided into four groups matched for mean body weight. Animals were either FD for 48 h or maintained on ad libitum feeding, primed with EB and P as before, and tested for sexual behavior. Three hours before the behavior test, all animals were pretreated with either saline or the type 1 GnRH receptor antagonist, Antide (1 μg/5 μl). This dose of Antide was selected based on the results of the Antide dose-response LH studies described above. Although a potent type 1 GnRH receptor antagonist, Antide possesses neither agonist nor antagonist activity at the type 2 GnRH receptor (23). Twenty minutes before the behavior test, an icv infusion of either GnRH II (1 μg) or saline was given to each female (n = 8 per group). See Table 2 for summary of treatment groups.

**TABLE 1. Experiment 2 treatment groups and testing paradigm**

<table>
<thead>
<tr>
<th>Group</th>
<th>Feeding regimen</th>
<th>Infusion treatment</th>
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<tbody>
<tr>
<td>1</td>
<td>Ad libitum</td>
<td>Saline</td>
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<tr>
<td>2</td>
<td>Ad libitum</td>
<td>GnRH I</td>
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<tr>
<td>3</td>
<td>Ad libitum</td>
<td>GnRH II</td>
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<tr>
<td>4</td>
<td>48-h Food deprivation</td>
<td>Saline</td>
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<tr>
<td>5</td>
<td>48-h Food deprivation</td>
<td>GnRH I</td>
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<tr>
<td>6</td>
<td>48-h Food deprivation</td>
<td>GnRH II</td>
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In experiment 2, mice maintained on ad libitum feeding were given one baseline sexual behavior test without drug infusion. A week later the mice were retested after undergoing feeding and drug treatments. For this test, food deprivation occurred in half the animals; GnRH I (1 μg), GnRH II (1 μg), or saline was infused icv 20 min before testing. All animals were primed with EB and P before each behavior test (n = 12–13 per group).

**TABLE 2. Experiment 3 treatment groups**

<table>
<thead>
<tr>
<th>Experiment group</th>
<th>Feeding regimen</th>
<th>Pretreatment</th>
<th>Hormone treatment</th>
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<tr>
<td>1</td>
<td>Ad libitum</td>
<td>Antide</td>
<td>Saline</td>
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<tr>
<td>2</td>
<td>48-h Food deprivation</td>
<td>Antide</td>
<td>Saline</td>
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<tr>
<td>3</td>
<td>48-h Food deprivation</td>
<td>Saline</td>
<td>GnRH II</td>
</tr>
<tr>
<td>4</td>
<td>48-h Food deprivation</td>
<td>Antide</td>
<td>GnRH II</td>
</tr>
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Three groups of females were food deprived for 48 h before a behavioral test; 1 group remained on ad libitum feeding. Pretreatment with either Antide (1 μg) or saline occurred 3 h before behavioral testing; infusion of GnRH II (1 μg) or saline occurred 20 min before testing. All animals were primed with EB and P before the test (n = 8 per group).

**Statistical analyses**

Longitudinal group differences in female sexual behavior and body weights between different trials (in experiments 1 and 2) were analyzed using repeated measures ANOVA with food and/or drug treatments as the main factors. Group differences in female mating behavior within a given trial (after various food or infusion treatments in experiments 1–3) were analyzed using factorial ANOVA with experiment-specific treatment groups as between-subjects factor. Plasma LH levels (in the Antide dose-response study) were analyzed using one-way ANOVA. For all ANOVA analyses, post hoc comparisons were made using a Fisher’s protected least significant difference test with a significance level set at P < 0.05.

**Results**

**FD inhibits female sex behavior**

Ovariectomized female mice that received EB and P priming showed increasing sexual behavior correlated with increasing experience. In experiment 1, the LQ of sexually naive females was minimal on trial 1 but increased with each subsequent trial, reaching maximal levels by trial 4 (Fig. 1). Reduced food availability had a significant impact on LQ (P < 0.001). Short-term FD of 24 h and 48 h on trial 5 significantly decreased female sexual behavior (by 30% and 68%, respectively, relative to ad libitum-fed controls; P < 0.001; Fig. 1A). The inhibitory effects of FD on female mating

![Fig. 1. LQ of hormonally primed female mice maintained on ad libitum (AL) feeding except on trial 5, when several groups had FD for 24 or 48 h, as indicated by the shaded region (A), or received FD for 48 h and then refed (RF) for either 0 h, 3 h, or 8 h before testing (B). Females displayed increasing sexual behavior with increasing experience. FD significantly reduced mating behavior on trial 5.](image-url)
behavior were transient because a return to \textit{ad libitum} feeding restored LQ to control levels a week later. In experiment 1B, females displayed a similar increase in LQ over time with successive sex behavior trials; 48 h FD on trial 5 resulted in a significant decline in female receptivity, which was partially reversed by 3 h of refeeding ($P < 0.01$; Fig. 1B). In comparison, 8 h of refeeding of previously underfed females fully restored sexual behavior; the LQ of 8 h refed females was not different from that of \textit{ad libitum}-fed controls but was significantly higher than that of females refeed for only 3 h ($P < 0.001$; Fig. 1B).

Mean body weights did not vary significantly between any of the groups on trial 4 before FD (22–23 g for all groups) but were significantly reduced in animals undergoing subsequent FD on trial 5 ($P < 0.05$). None of the underfed groups’ body weights differed from each other on trial 5, but all were 10–14% less than \textit{ad libitum}-fed animals at that time ($P < 0.05$).

\textbf{GnRH II restores sex behavior in underfed females}

Before FD or peptide treatment, females in all groups had equivalent LQs. Subsequent FD significantly decreased LQ in animals treated with saline or GnRHI ($P < 0.01$; Fig. 2A). In contrast, GnRH II enhanced the mating behavior of underfed females; the LQ of food deprived females receiving GnRHI was significantly higher than that of underfed animals receiving GnRHI or saline ($P < 0.01$; Fig. 2A). Conversely, GnRH II infusion had no stimulatory effect on LQ in females that were \textit{ad libitum} fed; the LQ of these animals was not significantly different from \textit{ad libitum} animals given saline (Fig. 2B). In contrast, GnRH I, which had no effect in underfed animals, resulted in a moderate but significant increase in LQ in \textit{ad libitum}-fed females, relative to the saline and GnRH II groups ($P < 0.05$; Fig. 2B).

Mean body weights were similar in all groups before FD (23–24 g); body weights of females that were subsequently food deprived were reduced by 12–14% relative to previous, \textit{ad libitum}-fed values ($P < 0.05$). Body weights of underfed females receiving GnRHI did not vary from those receiving saline or GnRHI.

\textbf{Blockade of type 1 GnRH receptor reduces plasma LH}

Central infusion (icv) of Antide, a type 1 GnRH receptor antagonist, reduced plasma LH concentration in a dose-dependent manner in ovariectomized female mice (Fig. 3A). The highest dose of Antide tested, 1.0 $\mu$g, reduced LH levels in plasma by almost 90% relative to saline administration ($P < 0.001$; Fig. 3A). The lower dose of Antide, 0.1 $\mu$g, was less effective but still reduced LH levels by 40% relative to saline infusions ($P < 0.01$). An Antide dose of 1.0 $\mu$g was also effective in preventing a GnRHI-induced LH surge in steroid-primed, ovariectomized females (Fig. 3B; $P < 0.001$ relative to saline-infused controls).

\textbf{Blockade of type 1 GnRH receptor fails to prevent GnRHI’s effects on behavior}

Pretreatment with Antide (1.0 $\mu$g) did not prevent GnRHI’s restorative effects on mating in underfed females (Fig. 4).

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig2.png}
\caption{LQ of hormonally primed female mice tested before and after GnRHI or saline treatment. A, All groups were \textit{ad libitum} (AL) fed on the first trial and food deprived for 48 h before the next test. GnRHI I or II (1 $\mu$g) was given 20 min before testing on the second trial. *, Significantly different from underfed saline and GnRHI I groups, as well as all groups in the baseline trial. B, GnRHI I or II (1 $\mu$g) or saline was given to \textit{ad libitum}-fed females 20 min before testing on the second trial. *, Significantly different from saline and GnRHI II groups on that trial.}
\end{figure}

Food-deprived females pretreated with Antide displayed low levels of mating when given saline before testing but showed a robust increase in LQ when given GnRHI II ($P < 0.001$; Fig. 4); the LQ of underfed females given Antide and then GnRHI II did not differ from underfed females pretreated with saline and then given GnRHI II. Although 10% lower than the LQ of \textit{ad libitum}-fed animals given Antide and then saline, the LQs of the two underfed groups given GnRHI II were not statistically different from the \textit{ad libitum} group ($P = 0.10$). Body weights of the three underfed groups were not significantly different from each other but were lower than those of the \textit{ad libitum}-fed group ($P < 0.01$).

\section*{Discussion}

Gonadotropin-releasing hormone was first isolated in the mammalian hypothalamus and shown to regulate the reproductive system by stimulating the release of pituitary gonadotropins. However, it is now apparent that GnRHI is but one of multiple structural variants that have evolved from a more ancient form (28). Here we report that the most primitive and universal form of GnRHI, GnRHI II, promotes sexual behavior in female mice. Moreover, we show for the
first time that the behavioral actions of GnRH II are not mediated by the type 1 GnRH receptor. We propose that GnRH II evolved to coordinate reproductive behaviors based on an animal’s energetic status. The primary function of GnRH II, therefore, appears to be as a neurochemical mediator between exogenous environmental conditions (availability of energy resources) and endogenous neuroregulatory processes (activation of sexual behavior).

A single GnRH II infusion administered icv significantly increased mating behavior in food-deprived female mice. In comparison, GnRH I had no stimulatory effect on lordosis behavior in underfed females. The lordosis quotient of underfed females given GnRH II was more than 30% higher than that of saline- or GnRH I-infused animals. Conversely, in ad libitum-fed females, GnRH II did not further elevate lordosis behavior above the level of sexual behavior observed in saline-infused control animals. This lack of GnRH II-mediated increase in lordosis in ad libitum-fed females is not simply due to lordosis already being maximal because GnRH I infusion further increased lordosis behavior in similarly fed females. These findings indicate that GnRH II can rapidly and significantly promote sexual behavior in females that would otherwise show minimal receptivity. However, because GnRH II does not have additional stimulatory effects in ad libitum-fed females, its actions are apparently permissive to reproduction, rather than merely stimulatory. Thus, under conditions when food is readily available, GnRH II is likely released at a basal rate that permits mating; in contrast, when energy status becomes compromised, GnRH II release decreases, temporarily disallowing the expression of female receptive behavior.

This is the first report of regulatory effects of GnRH II on sexual behavior in a rodent. Temple et al. (20) reported similar findings in the primitive mammalian species, the musk shrew (Suncus murinus). In these animals, similar to our findings in mice, GnRH II reverses the inhibitory effects of low food availability on female reproductive behavior but has little stimulatory effects in ad libitum-fed females (45). Interestingly, shrews, unlike mice, are induced ovulators that do not display hormonal or behavioral estrous cycles. Our present findings in mice therefore extend the permisive effects of GnRH II on sexual behavior to another order of mammals and to species that display estrous cycles and spontaneous ovulation. Numerous other mammals, including primates and humans, possess the GnRH II peptide in the brain (16, 49); it is intriguing to speculate that GnRH II might have similar regulatory actions on reproductive behavior in these species as well.

GnRH II peptide has been identified in rodent brains and immortalized mouse hypothalamic cell lines using RIA, HPLC, and immunohistochemical analyses (11–13, 50); these data, however, remain controversial because the gene for the GnRH II peptide has not yet been identified in the mouse genome (16) and several earlier studies were unable to find the peptide in brain (9, 51). Discrepancies in the rodent literature regarding the presence of GnRH II peptide may, in part, be due to the fact that most studies were performed in ad libitum-fed animals. In musk shrews, GnRH II release and storage appear to be contingent on food availability, with increased immunoreactivity revealed in GnRH-II containing...
neurons of underfed animals (20). Based on these findings, reexamination of the localization (and function) of GnRH II in rodents should be undertaken with animals that are on food restricted or food-deprived regimens. In support of this, we show here that GnRH II has substantial behavioral effects in underfed mice but minimal observable effects on behavior in ad libitum-fed mice.

Two GnRH receptors, type 1 and type 2, have been identified in mammals (17, 18, 49). Although GnRH II can bind both receptors, it has a 24-fold higher affinity for the type 2 compared with the type 1 GnRH receptor (17). It is unlikely that GnRH II’s permissive effects on reproductive behavior are mediated through the type 1 GnRH receptor because GnRH I, which binds this receptor with high affinity, had no positive effect on lordosis behavior in underfed mice (present study) or underfed female musk shrews (20). Furthermore, pretreatment of underfed female mice with Antide, a potent type 1 GnRH receptor antagonist, did not prevent the restorative effects of GnRH II infusion on sexual behavior. These findings indicate that GnRH II’s actions on behavior are likely mediated via the type 2 GnRH receptor or other as-yet unidentified GnRH receptors. In contrast, GnRH II’s stimulation of gonadotropin release in mammals is evidently mediated via the type 1 GnRH receptor because blocking this receptor prevents any GnRH II-induced LH release in both sheep and primates (23, 24). Development of type 2 GnRH receptor antagonists will better elucidate which receptor(s) GnRH II is binding to achieve its effects on sexual behavior.

The gene and transcripts of the type 2 GnRH receptor have been identified in the brains of multiple mammalian species, including musk shrews, pigs, marmosets, and rhesus monkeys (17–20, 52). Although ICC analysis has confirmed the presence of the type 2 receptor protein in mouse brain (17), the gene encoding the type 2 receptor in mouse has not yet been elucidated, and there is some evidence that this gene has been disrupted in several mammalian species, including sheep, rats, and humans (16). However, several studies have reported the expression of type 2 GnRH receptor mRNA in human cells and have described effects of GnRH II in human tissue even in the presence of type 1 GnRH receptor blockers (53–56). These findings indicate that GnRH II in humans is not acting through type 1 receptors, even though the type 2 receptor gene in humans appears to be incomplete (16). This raises the possibility that the disrupted type 2 receptor gene in humans and other species is able to encode a truncated but functional receptor. Alternatively, the type 2 receptor may be encoded for by a different gene that has yet to be identified. Lastly, it is possible that the effects of GnRH II may be mediated by a receptor that has yet to be discovered or by an orphan receptor that is already known. Future genomic and molecular studies will help to resolve these issues and determine which receptor(s) GnRH II binds to mediate its effects on sexual behavior in mice.

It is currently unknown which neural sites mediate GnRH II’s actions on female mating behavior in mammals. In musk shrews, the fibers of GnRH II-containing neurons are widespread and innervate numerous brain regions. Furthermore, type 2 GnRH receptors are located throughout the brain (17, 20, 49); icv infusion of GnRH II could potentially activate many of these regions. However, the best candidate sites are those containing type 2 GnRH receptors that have previously been implicated in the regulation of mammalian reproduction. In musk shrews and primates, type 2 GnRH receptors are present in the ventromedial hypothalamus, medial preoptic area, paraventricular nucleus, and the medial habenula (20, 49). One study has reported the presence of type 2 GnRH receptors in mouse pituitary (17); it remains to be determined where else in the mouse brain functional type 2 receptors are present, if at all. Current studies invoking cFos immunocytochemistry are underway in our lab to determine which neural sites are specifically activated by GnRH II infusion.

Although previous studies have reported the inhibitory effects of low food availability on estrous cycles and ovulation in mice, the present study is the first to document how energetic challenges impact mouse female sexual behavior. Similar to findings in hamsters and rats (39, 40, 57), short-term FD in the present study significantly reduced female sexual behavior in mice. The inhibitory effects of FD on lordosis were reversed by brief refeeding of 3–8 h. A similar time course of reinstatement of estrous behavior was reported for underfed female Syrian hamsters that were refeed for 3–6 h (41). In musk shrews, refeeding reinstates receptivity in underfed females in just 90 min (44). Collectively, these findings indicate that the refeeding cues that restore sexual behavior are detected and processed relatively quickly (within a few hours). The precise mechanism(s) and signals that mediate this process are unknown but likely involve a complex interaction of regulatory hormones and neurotransmitters (42, 57, 58). We propose that the GnRH II system is one of the key factors involved in this mediation of food availability on sexual behavior, as evidenced by the ability of GnRH II infusions to mimic the rapid restorative effects of brief refeeding on mating in underfed females. Furthermore, the type 2 GnRH receptor, in contrast to the type 1 receptor, possesses a cytoplasmic C-terminal tail that allows for fast desensitization and down-regulation (17, 18, 59); such rapid down-regulation of type 2 receptors may be an additional mechanism by which the GnRH II system quickly responds to rapid fluctuations in environmental energy availability.

In summary, energetically challenged mice showed enhanced female receptivity with treatment of GnRH II, but not GnRH I. GnRH I and II apparently modulate mammalian reproduction in different but complementary ways: GnRH I stimulates LH/FSH secretion and promotes lordosis behavior in ad libitum-fed animals, whereas GnRH II acts as a permissive gate, allowing reproductive behavior only when sufficient energy is available to support successful pregnancy and lactation. It is possible that GnRH II plays a similar role in regulating the behavior of humans, in which under-nutrition and energetic challenges reportedly dampen several aspects of female reproduction, including sexual desire and fertility (57, 60–62). GnRH II has already been localized in human brain and transcriptional and translational effects of the peptide have been observed in various human cell lines. A definitive test of the existence of a full-length, functional type 2 GnRH receptor in human brain will lend further support to the possibility that GnRH II plays a behavioral role in women.
Acknowledgments
We thank Aileen Wills and Harriet Sandridge for expert animal care, and Dr. Elka Scordalakes for surgical assistance.

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This work supported by National Institute of Mental Health Grants KO2 MH01349 and R01 MH68729 (E.F.R.), and by National Institutes of Health (NIH) Grant T32-HD07382 (A.S.K.). This research was also supported by National Institute of Child Health and Human Development/NIH through cooperative agreement U54 HD28934 as part of the Specialized Cooperative Centers Program in Reproduction Research.

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