REVIEW ARTICLE

# Control of Gonadotropin Secretion by Follicle-Stimulating Hormone-Releasing Factor, Luteinizing Hormone-Releasing Hormone, and Leptin

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Fractionation of hypothalamic extracts on a Sephadex G-25 column separates follicle-stimulating hormone-releasing factor (FSHRF) from luteinizing hormone-releasing hormone (LHRH). The FSH-releasing peak contained immunoreactive lamprey gonadotropin-releasing hormone (lGnRH) by radioimmunoassay, and its activity was inactivated by an antiserum specific to lGnRH. The identity of lGnRH-III with FSHRF is supported by studies with over 40 GnRH analogs that revealed that this is the sole analog with preferential FSH-releasing activity. Selective activity appears to require amino acids 5–8 of lGnRH-III. Chicken GnRH-II has slight selective FSH-releasing activity. Using a specific lGnRH-III antiserum, a population of lGnRH-III neurons was visualized in the dorsal and ventral preoptic area with axons projecting to the median eminence in areas shown previously to control FSH secretion based on lesion and stimulation studies. Some lGnRH-III neurons contained only this peptide, others also contained LHRH, and still others contained only LHRH. The differential pulsatile release of FSH and LH and their differential secretion at different times of the estrous cycle may be caused by differential secretion of FSHRF and LHRH. Both FSH and LHRH act by nitric oxide (NO) that generates cyclic guanosine monophosphate. lGnRH-III has very low affinity to the LHRH receptor. Biotinylated lGnRH-III ($10^{-9}$ M) labels 80% of FSH gonadotropes and is not displaced by LHRH, providing evidence for the existence of an FSHRF receptor. Leptin has equal potency as LHRH to release gonadotropins by NO. lGnRH-III specifically releases FSH, not only in rats but also in cows.   © 2001 IMSS.   Published by Elsevier Science Inc.

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## Introduction

Control of gonadotropin secretion is extremely complex, as revealed by the research of the past 40 years since the discovery of luteinizing hormone-releasing hormone (LHRH) (1), now commonly called gonadotropin-releasing hormone (GnRH) (2). This was the second of the hypothalamic-releasing hormones characterized. It stimulates follicle-stimulating hormone (FSH) release, albeit in smaller amounts than luteinizing hormone (LH). For this reason, it was renamed GnRH (2,3). Overwhelming evidence indicates that there must be a separate FSH-releasing factor (FSHRF) because pulsatile release of LH and FSH can be dissociated. In the castrated male rat, roughly one half of FSH pulses occur in the absence of LH pulses and only a small fraction of the pulses of both gonadotropins are coincident. LHRH antisera or antagonists can suppress pulsatile release of LH without altering FSH pulses (4,5). LH but not FSH pulses can be suppressed by alcohol (6), delta-9-tetrahydrocannabinol, and cytokines such as interleukin-1 alpha (IL-1α) (7). In addition, a number of peptides inhibit LH but not FSH release, and a few stimulate FSH without affecting LH (4,8).

The hypothalamic areas controlling LH and FSH are separable. Stimulation in the dorsal anterior hypothalamic area causes selective FSH release, whereas lesions in this area selectively suppress the pulses of FSH and not LH (9). Conversely, stimulations or lesions in the medial preoptic region can augment or suppress LH release, respectively, without affecting FSH release. Electrical stimulation in the preoptic region releases only LH, whereas lesions in this area inhibit LH release without inhibiting FSH release. The medial preoptic area contains the majority of the perikarya of LHRH neurons. The axons of these neurons project from the preoptic region to the anterior and mid-portions of the median eminence. Extracts of the anterior mid-median eminence contain LH-releasing activity commensurate with the content of immunoassayable LHRH, whereas extracts of the caudal-median eminence and organum vasculosum lamina terminalis (OVLT) contain more FSH-releasing activity than can be accounted for by the content of LHRH (4).

Lesions confined to the rostral and mid-median eminence can selectively inhibit pulsatile LH release without altering FSH pulsations, whereas lesions that destroy the caudal and mid-median eminence can selectively block FSH pulses in castrated male rats (4,9,10). Therefore, the putative FSHRF may be synthesized in neurons with perikarya in the dorsal anterior hypothalamic area with axons that project to the mid- and caudal-median eminence to control FSH release selectively.

## FSHRF

We (followed by several other groups) reported FSH-releasing activity in the stalk-median eminence (11). The activity was purified and separated from LH-releasing activity in 1965 *in vivo* as measured by bioassays (12,13). This separation was confirmed by three additional laboratories (14). It has been shown repeatedly that bioactive and radioimmunoassayable FSH-releasing activity can be separated from LHRH by gel filtration through Sephadex G-25 on the same column used in the earlier research. FSH- and LH-releasing activity were assayed by the increase in plasma FSH and LH, respectively, in ovariectomized, estrogen progesterone-blocked rats (15). Separation of the two activities was also demonstrable by assay of FSH and LH released from hemipituitaries incubated *in vitro* by bioassay (16) and RIA (17). In both assay systems, FSHRF emerged from the column immediately before elution of LHRH.

In the search for FSHRF, we first believed that it might be an analog of LHRH (12) and later had many such analogs synthesized. We tested the forms of GnRH known to exist in lower species (Yu et al., in preparation). We had not tested lamprey (l) GnRH-III, but when we realized that an antiserum that cross-reacted with lGnRH-I and lGnRH-III immunostained neural fibers in the arcuate nucleus proceeding to the median eminence of human brains obtained at autopsy, it occurred to us that lGnRH-III could be the FSHRF, because lGnRH-I had little activity for release of either LH or FSH (18). Indeed, lGnRH-III is a potent FSH-releasing factor with little or no LH-releasing activity both *in vitro* when incubated with hemipituitaries of male rats and *in vivo* when injected into conscious, ovariectomized, estrogen progesterone-blocked rats (19). The lowest dose tested in that preparation (10 pmol) produced a highly significant increase in plasma FSH with no increase in LH; a 10-fold higher dose (100 pmol) increased plasma FSH similarly and had no effect on LH release. lGnRH-III $10^{-9}$ M produced highly significant FSH release *in vitro*, whereas LH-releasing activity did not appear until $10^{-6}$ M (19).

Testing other GnRH analogs in this *in vitro* system revealed that chicken (c) GnRH-II had no significant activity to release FSH or LH until a much higher dose was reached and no selective releasing activity. It had slightly selective FSH-releasing activity *in vivo* in ovariectomized, estrogen progesterone-blocked rats (18). We tested over 40 natural and synthesized analogs of lGnRH-III and found that the amino acids in positions 5, 6, 7, and 8 of lGnRH-III are important in conveying selective FSH-releasing activity. These four amino acids are dissimilar from those in LHRH. From tunicate to man, the first four amino acids in GnRHs are nearly constant and the last two positions, 9 and 10, are constant. This leads us to conclude that amino acids 5–8 are crucial for determining whether or not an analog will be selective for FSH or LH release (Yu et al., in preparation).

We fractionated 1,000 rat hypothalami by gel filtration on Sephadex G-25, determined the FSH- and LH-releasing activity of the various fractions by bioassay on male rat hemipituitaries, and compared the localization of these activities with that of LHRH and lGnRH determined by RIA. lGnRH was assayed by RIA using a specific antiserum for lGnRH that recognized lGnRH I and III equally but did not cross-react with LHRH or cGnRH-II. A peak of LHRH immunoreactivity was found as well as three peaks of lGnRH immunoreactivity that preceded the peak of LHRH. The first peak eluted was quite small. The second peak was much larger and the third peak was of the greatest magnitude. Only the second peak altered gonadotropin release and produced selective FSH release.

To determine whether this activity was caused by lGnRH, anterior hemipituitaries were incubated with normal rabbit serum or the lGnRH antiserum (1:1,000); the effect on FSH and LH-releasing activity of the FSH-releasing fraction and the LH-releasing activity of LHRH was determined. The antiserum had no effect on basal release of either FSH or LH, but eliminated the FSH-releasing activity of the active fraction without altering the LH-releasing activity of LHRH. Because lGnRH-1 has minimal nonselective activity to release FSH or LH whereas previous experiments showed that lGnRH-III highly selectively releases FSH with a potency equal to that of LHRH to release LH, these results support the hypothesis that the FSH-releasing

activity observed in the FSHRF fraction was caused by lGnRH-III or a very closely related peptide (17).

## Localization of lGnRH in the Brain of Rats by Immunocytochemistry

Using the same antiserum that we employed to find the location of lGnRH after gel-filtration of rat hypothalami, we attempted to localize lGnRH neurons by immunocytochemistry. Immunoreactive lGnRH-like cell bodies were found in the ventromedial preoptic area with axons projecting to the rostral wall of the third ventricle (3V) and organum vasculosum lamina terminalis (OVLT). Another population of lGnRH-like cell bodies was located in the dorsomedial preoptic area with axons projecting caudally and ventrally to the external layer of the median eminence. On the other hand, using a highly specific monoclonal antiserum against mammalian (m) GnRH to localize the mGnRH neurons so that their localization could be compared with that of lGnRH neurons, we found that there were no mGnRH cells in the dorsomedial caudal preoptic area that contained perikarya and fibers of lGnRH neurons (20).

Furthermore, immunoabsorption studies indicated that the cell bodies of the lGnRH neurons were eliminated by lGnRH-III but not by mGnRH, whereas the axons in the median eminence were eliminated by lGnRH-III but only slightly reduced by absorption with mGnRH. Using an antiserum against cGnRH-II that visualized cGnRH-II neurons in the chicken hypothalamus, no such neurons could be visualized in the rat hypothalamus (20).

Because the lGnRH antiserum (#3952) recognized lGnRH-I and lGnRH-III equally, a specific antiserum against lGnRH-III without cross-reactivity with lGnRH-I was needed to prove that the lGnRH neurons visualized in the rat brain were indeed lGnRH-III neurons. Our recent studies indicate that the specific lGnRH-III antiserum (#39-82-78-3) visualizes the same population of neurons observed with the less specific lGnRH antiserum (#3952). Additionally, the staining of cells and fibers could be eliminated by lGnRH-III but was only slightly affected by lGnRH-I or mGnRH. Consequently, these results strongly support the hypothesis that the lGnRH-III neurons located in the areas of the brain responsible for control of FSH are the FSHRF neurons (Hiney et al., in preparation). The lGnRH-III neurons whose cell bodies are located in the caudal dorso-medial preoptic area with axons projecting to the median eminence are in the very regions shown to be selectively involved in the control of FSH release by lesion and stimulation studies described earlier. Lesions in the caudal dorsal medial preoptic and anterior dorsal medial anterior hypothalamic area blocked the castration-induced rise in FSH (21) and blocked pulsatile FSH release without interfering with pulsatile LH release (22). Conversely, implants of prostaglandin $E_2$ (PGE$_2$) in this region evoked selective FSH release. These evoked a pattern of FSH-selective release from implants of PGE$_2$ along a path running from the medial dorsal preoptic area to the caudal parts of the median eminence regions (23) shown to contain more FSH-releasing activity than could be accounted for by LHRH, and that when destroyed can also selectively block FSH and not LH pulses in castrated male rats (10).

The remaining population of lGnRH-III neurons in the ventromedial caudal preoptic area has axons that appear to project to the OVLT and to the wall of the 3V, whereas the more abundant dorsal neurons also project to the ventricle and caudally to the mid-brain central gray along the same pathway as the LHRH neurons (19). Projection to mid-brain central gray suggests the possibility that lGnRH-III may be involved in mating behavior, in that this is the area that LHRH activates to induce mating behavior.

The function of the caudal ventral medial preoptic neurons projecting to the OVLT is not clear. Possibly, they release lGnRH-III into the ventricular system for actions more caudally subsequent to its uptake from the ventricle. Alternatively, their axons may in some manner also reach the median eminence via an unknown trajectory. It is interesting that some of these neurons in this region also contain mLHRH, and that there are other neurons therein that contain only LHRH. These are located laterally to the positions of the lGnRH-III neurons that are more medial. The fact that some of the neurons contain both peptides suggests that they may interact in an ultra short-loop feedback mechanism to control the release not only of FSHRF, but also LHRH.

## Evidence for a Specific FSHRF Receptor

The selectivity of release of FSH by FSHRF and lGnRH-III suggests the probability of a specific FSHRH receptor on pituitary gonadotropes. In an early experiment in collaboration with J. Rivier and W. Vale (Salk Institute, La Jolla, CA, USA), we tested the FSH-releasing activity of our purified FSHRF using monolayered cultured pituitary cells and were unable to detect any FSH-releasing activity (Yu et al., unpublished data). This led us to the hypothesis that the FSHRF receptors are downregulated during the 4 days in culture in the absence of gonadal steroids. Indeed, in a recent experiment we confirmed the fact that lGnRH-III has little activity on monolayered cultured male rat pituitary cells at the same time that LHRH is active at $10^{-10}$ M. lGnRH-III was also tested in a LHRH receptor assay. The production of inositol phosphate in COS cells transfected with LHRH receptors was not increased by lGnRH-III until a concentration of $10^{-7}$ M was reached. As the concentration was increased, full activity was obtained at $10^{-4}$ M (10).

We hypothesized that we might demonstrate the presence of lGnRH-III receptors on gonadotropes using biotinylated lGnRH-III and mLHRH. To enhance imaging, an avidin-based system was used combined with immunocytochemistry for FSH and LH. The lGnRH-III peptide was

extended with one or two spacer arms of aminocaproic acid between the biotin moiety and one amino acid of the peptide. Three biotinylated derivatives of lGnRH-III were tested for bioactivity. The peptide derivative biotinylated on Lys[8] with two spacer arms of aminocaproic acid (Bxx-Lys[8]-lGnRH-III) had selective FSH-releasing activity at a dose of $10^{-8}$ M on hemipituitaries incubated *in vitro*, whereas the analog with one spacer arm (Bx-Lys[8]) had less activity than that of the previously mentioned derivative. By contrast, the analog biotinylated on Asp[6] and one spacer arm (Bx-Asp[6]) had no selective FSH-releasing activity. Therefore, the two Bxx-lGnRH-III appeared to be satisfactory for locating lGnRH-III binding sites on pituitary gonadotropes. Biotinylated lGnRH-III ($10^{-9}$ M) bound to 80% of FSH gonadotropes and only 50% of LH gonadotropes of acutely dispersed pituitary cells, a finding that indicates that there are receptors on gonadotropes that bind this peptide (25). The binding of biotinylated lGnRH-III was not displaced with LHRH, indicating that it is highly specific for the putative FSHRF receptors. It appears that in this situation as with monolayer cultured pituitary cells, the FSHRF receptors disappear with time in culture because with 24 h in culture, the binding of the biotinylated lGnRH-III was significantly decreased. On the basis of our recent studies with biotinylated lGnRH-III, we believe that ultimately a specific receptor for this peptide will be found in the pituitary; additionally, it is in all probability the FSH-releasing factor.

lGnRH-III binds to the three GnRH receptors discovered in the bullfrog, but the affinity to the receptors was less than that of mLHRH or cGnRH-II as determined in the inositol phosphate assay (25,26). Neil et al. (27) recently reported the existence of a second GnRH receptor (GnRH-IIr) in the human genome and additionally reported the cloning and characterization of its cDNA from monkeys. The cDNA generated a G protein-coupled transmembrane receptor having a C-terminal cytoplasmic tail, whereas GnRH-Ir lacks this tail. GnRH-IIr more closely resembles the type II receptors of amphibians and fish than it does the type I receptor of humans. This receptor is specific for cGnRH-II and is expressed ubiquitously in human tissues (27).

Previously, cGnRH-II has been reported in various tissues in monkeys and humans, but the peptide was found in the hypothalamus of only fetal monkeys and also in the mid-brain central gray (28). In adult monkeys, there were only a few cells and fibers in the caudal hypothalamus, suggesting that this peptide would not reach the pituitary via the portal vessels but must have other actions perhaps on mating behavior or in regulation of cell division. Indeed, as indicated previously we did not find cGnRH-II in the hypothalamus of the rat, in contrast with the readily observed lGnRH-III neurons and terminals in the median eminence. Furthermore, cGnRH-II, as previously mentioned, has little or no selective FSH-releasing activity; however, there is little doubt that it is an ancient GnRH existing from fish to mammals.

## Mechanism of Action of FSHRF, LHRH, and Leptin on Gonadotropin Secretion

It is well known that FSH and LH release is controlled by calcium ions ($Ca^{++}$) (29,30) and that interaction of LHRH with its receptor causes an increase in intracellular-free calcium and also activates the phosphatidyl inositol cycle that mobilizes internal calcium. The resulting increase in intracellular-free calcium mediates the releasing action of LHRH (for review, see Reference 30); however, we earlier showed a role for a cyclic guanosine monophosphate (cGMP) and not cyclic adenosine monophosphate (cAMP) in controlling the release of LH and FSH mediated by LHRH (31–33). This was before it was accepted that nitric oxide (NO) is a physiologically significant gaseous transmitter that acts by activation of guanylyl cyclase that converts GTP to cGMP. cGMP activates protein kinase G that causes exocytosis of gonadotropin secretory granules.

To test the hypothesis that the FSH-releasing activity of lGnRH-III (or FSHRF) is mediated by calcium and NO, calcium was removed from the medium and a chelating agent (ethylene glycol-N-N-N′-N′-tetraacetic acid) that would remove any residual $Ca^{++}$ was added. The action of FSHRF and lGnRH-III was blocked in the absence of $Ca^{++}$. $N^G$-mono-methyl-L-arginine (NMMA), a competitive inhibitor of NO synthase (NOS), was added to the medium in other experiments. We found that the inhibitor of NOS, NMMA, completely blocked the FSH-releasing activity of not only purified FSHRF but also of lGnRH-III. Furthermore, sodium nitroprusside (NP), a releaser of NO, stimulated both LH and FSH release and the activity of LHRH to release both LH and FSH was also blocked by NMMA. These data indicate that FSHRF (or lGnRH-III) acts on its putative receptor via a calcium-dependent nitric oxide pathway to release FSH specifically, whereas LHRH acts similarly on its receptor to increase intracellular $Ca^{++}$ that activates NOS in the gonadotropes to cause release of LH and to a lesser extent, FSH (24,34).

## Possible Use of lGnRH-III in Control of Reproduction

Not only does lGnRH-III have selective FSH-releasing activity *in vitro* but also *in vivo* in the ovariectomized, estrogen progesterone-blocked rat (19) and normal male rat (Yu et al., unpublished), but also in the cycling cow (35). Furthermore, treatment of cows with lGnRH-III can produce multiple large follicles, in striking contrast to the normal development of a single large follicle in each cycle. On the basis of these findings, we believe that lGnRH-III has great potential to alter reproduction in animals and man.

## Role of NO in Control of LHRH Release

NO is formed in the body by NOS, an enzyme that converts arginine in the presence of oxygen and several cofactors into equimolar quantities of citrulline and NO. There are three isoforms of the enzyme. One, neural (n)NOS, is found

in the cerebellum and various regions of the cerebral cortex and additionally in various ganglion cells of the autonomic nervous system. Large numbers of nNOS-containing neurons, termed NOergic neurons, were also found in the hypothalamus, particularly in the paraventricular and supraoptic nuclei with axons projecting to the median eminence and neural lobe, which also contains large amounts of nNOS. These findings indicated that the enzyme is synthesized at all levels of the neuron from perikarya to axon terminals (36).

Because of this distribution in the hypothalamus in regions that contain peptidergic neurons that control pituitary hormone secretion, we decided to determine the role of this soluble gas in the release of LHRH. The approach employed was to use sodium NP that spontaneously liberates NO to see whether this altered the release of various hypothalamic transmitters. Hemoglobin, which scavenges NO by a reaction with the heme group in the molecule and inhibitors of NOS such as NMMA, a competitive inhibitor of NOS, was used to determine the effects of decreased NO. Two types of studies were performed. In the first set of experiments, medial basal hypothalamic (MBH) explants were preincubated *in vitro* and then exposed to neurotransmitters that modify the release of various hypothalamic peptides in the presence or absence of inhibitors of the release of NO. The response to NO itself, provided by sodium NP, was also evaluated. To determine whether the results *in vitro* also held *in vivo*, substances were microinjected into the 3V of the brain of conscious, freely moving animals to determine the effect on pituitary hormone release (37).

Our most extensive studies were carried out with regard to the release of LHRH. Not only does LHRH act after its secretion into the hypophyseal portal vessels to stimulate LH and to a lesser extent FSH release, but it also induces mating behavior in female rats and penile erection in male rats by hypothalamic action (38).

Our experiments showed that release of NO from sodium NP *in vitro* promoted LHRH release and that the action was blocked by hemoglobin, a scavenger of NO. NP also caused an increased release of $PGE_2$ from the tissue, which previous experiments showed played an important role in release of LHRH. Furthermore, it caused the biosynthesis and release of prostanoids from $^{14}C$ arachidonic acid. The effect was most pronounced for $PGE_2$, but there also was release of lipoxygenase products, which have been shown to play a role in LHRH release. Inhibitors of cyclooxygenase, the enzyme responsible for prostanoid synthesis, such as indomethacin and salicylic acid, blocked the release of LHRH induced by NE, providing further evidence for the role of NO in the control of LHRH release via the activation of cyclooxygenase-1. Nedleman's group later showed that NO activates cyclooxygenase-1 and cyclooxygenase-2 in cultured fibroblasts. The action is probably mediated by combination of NO with the heme group of cyclooxygenase, altering its conformation. The action on lipoxygenase is similar; although it contains ferrous iron, the actual presence of heme in lipoxygenase has yet to be demonstrated (37,39).

As indicated, the previously accepted pathway for the physiologic action of NO is by activation of soluble guanylate cyclase by means of interaction of NO with the heme group of this enzyme, thereby causing conversion of guanosine triphosphate into cGMP, which mediates the effect on smooth muscle by decreasing the intracellular $[Ca^{++}]$. On the other hand, Muelom's group showed in incubated pancreatic acinar cells that cGMP has opposite effects on intracellular $[Ca^{++}]$, elevating it at low concentrations and lowering it at higher concentrations. We postulate that the NO released from the NOergic neurons near the LHRH neuronal terminals increases the intracellular free calcium required to activate phospholipase $A_2$ ($PLA_2$). $PLA_2$ causes the conversion of membrane phospholipids in the LH terminal into arachidonate, which then can be converted into $PGE_2$ via the activated cyclooxygenase. The released $PGE_2$ activates adenyl cyclase causing an increase in cAMP release, which activates protein kinase-A, leading to exocytosis of LHRH secretory granules into the hypophyseal portal capillaries for transmission to the anterior pituitary gland (40).

Norepinephrine (NE) has previously been shown to be a powerful releaser of LHRH. It acts by activation of the NOergic neurons because the activation of these neurons and the release of LHRH could be blocked by a competitive inhibitor of NOS, NMMA. NE acts to stimulate the release of NO from the NOergic neurons by $\alpha_1$ adrenergic receptors because its action can be blocked by phentolamine, an $\alpha$ receptor blocker, and prazosine, a specific $\alpha_1$ receptor blocker. Activation of the $\alpha_1$ receptors is postulated to increase the intracellular $[Ca^{++}]$ that combines with calmodulin to activate NOS leading to generation of NO.

We measured the effect of NE on the content of NOS in the MBH explants at the end of the experiments by homogenizing the tissue and adding $^{14}C$ arginine and measuring its conversion to citrulline on incubation of the homogenate. Because arginine is converted into equimolar quantities of NO and citrulline, measurement of citrulline production provides a convenient estimate of the activity of the enzyme. NO disappears rapidly, making its measurement very difficult. NE caused an increase in the apparent content of the enzyme. That we were actually measuring enzyme content was confirmed, because incubation of the homogenate with L-nitroargine methyl ester, another inhibitor of NOS, caused a drastic decline in the conversion of arginine into citrulline. We further confirmed that we actually had increased the content of enzyme by isolating the enzyme according to the method of Bredt and Snyder (41) and then measuring the conversion of labeled arginine into citrulline. The conversion was increased to a greater degree by NE (42).

Glutamic acid (GA), at least in part by n-methyl-D-aspartate receptors, also plays a physiologically significant role in controlling the release of LHRH. Therefore, we evaluated

where GA fits into the picture. It also acted via NO to stimulate LHRH release, but we showed that the effect of GA could be completely obliterated by the α-receptor blocker phentolamine. Consequently, we concluded that GA acted by stimulation of the noradrenergic terminals in the MBH to release NE, which then initiated NO release and stimulation of LHRH release (43).

Oxytocin has actions within the brain to promote mating behavior in the female and penile erection in the male rat. Because LHRH mediates mating behavior, we hypothesized that oxytocin would stimulate the LHRH release that, after secretion into the hypophyseal portal vessels, mediates LH release from the pituitary. Consequently, we incubated MBH explants and demonstrated that oxytocin ($10^{-7}$–$10^{-10}$ M) induced LHRH release via NE stimulation of nNOS. Therefore, oxytocin may be very important as a stimulator of LHRH release. Furthermore, NO acted as a negative feedback to block oxytocin release (44).

One of the few receptors identified on LHRH neurons is the gamma amino butyric acid a (GABAa) receptor. Consequently, we evaluated the role of GABA in LHRH release and the participation of NO in same. Experiments showed that GABA blocked the response of LHRH neurons to NP that acts directly on the LHRH terminals. We concluded that GABA suppressed LHRH release by blocking their response to NO. Additional experiments showed that NO stimulates the release of GABA, thereby providing an inhibitory feedforward pathway to inhibit the pulsatile release of LHRH initiated by NE. As NE stimulated the release of NO, this would stimulate the release of GABA, which would then block the response of the LHRH neuron to the NO released by NE (45).

Other studies indicated that NO would suppress the release of dopamine and NE. We have already described the ability of NE to stimulate LHRH; dopamine also acts as a stimulatory transmitter in the pathway. Therefore, there is an ultra short-loop negative feedback mechanism to terminate the pulsatile release of LHRH because the NO released by NE would diffuse to the noradrenergic terminals and inhibit release of NE, thereby terminating the pulse of NE, LHRH, and finally LH (46).

We further examined the possibility that other products from this system might have inhibitory actions. Indeed, we found that as we added increasing amounts of NP we obtained a bell-shaped, dose-response curve of the release of LHRH, such that the release increased with increasing concentrations of NP up to a maximum at approximately 600 μM and then declined with higher concentrations. When the effect of NP on NOS content at the end of the experiment was measured, we found that high concentrations of NP lowered the NOS content. Furthermore, NP could directly decrease NOS content when incubated with MBH homogenates, results that indicate a direct effect on NOS probably by interaction of NO with the heme group on the enzyme. Thus, when large quantities of NO are released, as could oc-

cur after the induction of inducible (i)NOS in the brain during infections, the release of NO would decrease by an inhibitory action on the enzyme at these high concentrations. Furthermore, high concentrations of cGMP released by NO also acted in the explants or even in the homogenates to suppress the activation of NOS. This pathway could also be active in the presence of high concentrations of NO, such as would occur in infection by induction of iNOS by bacterial or viral products (42).

## The Effect of Cytokines (IL-1 and Granulocyte Macrophage Colony-Stimulating Factor [GMCSF]) on the NOergic Control of LHRH Release

The cytokines tested to date, for example IL-1 and GMCSF, act within the hypothalamus to suppress the release of LHRH as revealed in both *in vivo* and *in vitro* studies. We have examined the mechanism of this effect and found that for IL-1 it occurs by inhibition of cyclooxygenase as shown by the fact that there is blockage of the conversion of labeled arachidonate to prostanoids, particularly PGE$_2$, and the release of PGE$_2$ induced by NE is also blocked (47).

A principle mechanism of action is by suppression of LHRH release induced by NO donors such as NP. We first believed that there were IL-1 and GMCSF receptors on the LHRH neuron that blocked the response of the neuron to NO. However, because we had also shown that GABA blocks the response to NP and earlier work had shown that GABA receptors are present on the LHRH neurons, we evaluated the possibility that the action of cytokines could be mediated by stimulation of GABAergic neurons in the MBH. Indeed, in the case of GMCSF its inhibitory action on LHRH release can be partially reversed by the GABAa receptor blocker, bicuculline, which also blocks the inhibitory action of GABA itself on the response of the LHRH terminals to NO. Therefore, we believe that the inhibitory action of cytokines on LHRH release is mediated by stimulation of GABA neurons (48).

## Role of NO in Mating Behavior

LHRH controls lordosis behavior in the female rat and is also involved in mediating male sex behavior. Studies *in vivo* have shown that NO stimulates the release of LHRH involved in inducing sex behavior. This behavior can be stimulated by a third ventricular injection of NP and is blocked by inhibitors of NOS. Apparently, there are two LHRH neuronal systems: one with axons terminating on the hypophyseal portal vessels, the other with axons terminating on neurons that mediate sex behavior (38). NO is also involved in inducing penile erection by the release of NO from NOergic neurons innervating the corpora cavernosa penis. The role of NO in sex behavior in both sexes has led us to change the name of NO to the sexual gas (36).

## Potential Role of Leptin in Reproduction

The hypothesis that leptin may play an important role in reproduction stems from several findings. First, the Ob/Ob mouse, lacking the normal leptin gene, is infertile and has atrophic reproductive organs. Gonadotropin secretion is impaired and very sensitive to negative feedback by gonadal steroids, as is the case for prepubertal animals (49,50). It has now been shown that treatment with leptin can recover the reproductive system in the Ob/Ob mouse by leading to growth and function of the reproductive organs and fertility (51) via secretion of gonadotropins (52).

The critical weight hypothesis of the development of puberty states that when body fat stores have reached a certain point, puberty occurs (53). This hypothesis in its original form is not acceptable because if animals are underfed, puberty is delayed, but with access to food, rapid weight gain leads to the onset of puberty at weights well below the critical weight under normal nutritional conditions (54). We hypothesized that during this period of refeeding or at the time of critical weight in normally fed animals as fat stores increase, there is increased release of leptin from the adipocytes into the blood stream and this acts on the hypothalamus to stimulate the release of LHRH with resultant induction of puberty. Indeed, leptin increases LHRH in prepubertal rats (55). Whether leptin is initiating puberty or is a permissive factor in the process is debatable.

We initiated studies on its possible effects on hypothalamic-pituitary function. We anticipated that it would also be active in adult rats; therefore, we studied its effect on the release of FSH and LH from hemipituitaries and also its possible action to release LHRH from MBH explants *in vitro*. To determine whether leptin was active *in vivo*, we used a model that we have often employed to evaluate stimulatory effects of peptides on LH release: namely, the ovariectomized, estrogen-primed rat. Because our supply of leptin was limited, we began by microinjecting it into the 3V in conscious animals bearing implanted third ventricular cannulae and also catheters in the external jugular vein extending to the right atrium, so that we could draw blood samples prior and subsequent to injection of leptin and measure the effect on plasma FSH and LH (56).

## Effect of Leptin on Gonadotropin Release

We found leptin had a bell-shaped, dose-response curve to release LH from anterior pituitaries incubated *in vitro*. There was no consistent stimulation of LH release at a concentration of $10^{-5}$ M. Results became significant with $10^{-7}$ M and remained on a plateau through $10^{-11}$ M with reduced release at a concentration of $10^{-12}$ M that was no longer significant statistically. The release was not significantly less than that achieved with LHRH ($4 \times 10^{-8}$ M). Under these conditions, there was no additional release of LH when leptin ($10^{-7}$ M) was incubated together with LHRH ($4 \times 10^{-8}$ M). In certain other experiments, there was an additive effect when leptin was incubated with LHRH; however, this effect was not uniformly observed. Results indicate that leptin was only slightly less effective to release LH than LHRH itself (56).

## Effect of Leptin on FSH Release

In the incubates from these same glands, we also measured FSH release and found that it showed a similar pattern to that of LH, except that sensitivity in terms of FSH release was much less than that for LH. The minimal effective dose for FSH was $10^{-9}$ M, whereas for LH it was $10^{-11}$ M. The responses were roughly of the same magnitude as obtained with LH at the effective concentrations; in addition, the responses were clearly equivalent to those observed with $4 \times 10^{-9}$ M LHRH. A combination of LHRH with a concentration of leptin that was immediately below significance gave a clear additive effect (56).

## Effect of Leptin on LHRH Release

There was no significant effect of leptin in a concentration range of $10^{-6}$–$10^{-12}$ M on LHRH release during the first 30 min of incubation; however, during the second 30 min the highest concentration produced a borderline significant decrease in LHRH release with $10^{-6}$ M, followed by a tendency to increase with lower concentrations and a significant ($p < 0.01$) plateaued increase with the lowest concentrations tested ($10^{-10}$ and $10^{-12}$ M) (57). Both the FSH and LH-releasing actions of leptin were blocked by NMMA, indicating that NO mediates its action (34).

## The Effect of Intraventricularly Injected Leptin on Plasma Gonadotropin Concentrations in Ovariectomized, Estrogen-Primed Rats

The injection of the diluent for leptin into the 3V (Krebs-Ringer bicarbonate, 5 μL) had no effect on pulsatile FSH or LH release, but injection of leptin (10 μg) uniformly produced an increase in plasma LH with a variable time-lag ranging from 10 to 50 min, so that the maximal increase in LH from the starting value was highly significant ($p < 0.01$) and constituted a mean increase of 60% above the initial concentration. In contrast, leptin inhibited FSH release in comparison with the results with the diluent, but the effect was delayed and occurred principally during the second hour. Therefore, at this dose of estrogen (10 μg estradiol benzoates, 72 h before experiments), leptin stimulates the release of LHRH and inhibits the release of FSHRF (57).

## Mechanism of Action of Leptin on the Hypothalamic Pituitary Axis

We have shown that leptin exerts its action at both the hypothalamic and pituitary levels by activating NOS because its

effect to release LHRH (56), FSH, and LH *in vitro* (34) is blocked by NMMA (38). Leptin, in essence, is a cytokine secreted by the adipocytes. It, like the cytokines, appears to reach the brain via a transport mechanism mediated by the $Ob/Ob_a$ receptors (58) in the choroid plexus (59). These receptors have an extensive extracellular domain but a greatly truncated intracellular domain (58) and mediate transport of the cytokine by a saturable mechanism (60). Following uptake into the cerebrospinal fluid (CSF) through the choroid plexus, leptin is carried by the flow of CSF to the 3V, where it either diffuses into the hypothalamus through the ependymal layer lining the ventricle or combines with $Ob_b$ (58) receptors on terminals of responsive neurons that extend to the ventricular wall.

The $Ob_b$ receptor has a large intracellular domain that presumably mediates the action of the protein (59). These receptors are widespread throughout the brain (59) but are particularly localized in the region of the paraventricular (PVN) and arcuate nuclei (AN). Leptin activates stat 3 within 30 min after its intraventricular injection (61). Stat 3 is a protein important in conveying information to the nucleus to initiate DNA-directed mRNA synthesis. Following injection of bacterial lipopolysaccharide (LPS) it is also activated, but in this case the time delay is 90 min, presumably because LPS has been shown to induce IL-1βm RNA in the same areas, namely, the PVN and AN (59). IL-1β mRNA would then cause production of IL-1β that would activate stat 3. On entrance into the nucleus, stat 3 would activate or inhibit DNA-directed mRNA synthesis. In the case of leptin, it activates CRH mRNA in the PVN, whereas in the AN it inhibits neuropeptide Y (NPY) mRNA, resulting in increased CRH synthesis, and presumably release in the PVN and decreased NPY synthesis and release in the AN (59). Presumably, the combination of leptin with these transducing receptors also either increases or decreases the firing rate of that particular neuron. In the case of the AN-median eminence area leptin may enter the median eminence by diffusion between the tanycytes or alternatively by combining with its receptors on terminals of neurons projecting to the tanycytes. Activation or inhibition of these neurons would induce LHRH release. The complete pathway of leptin action in the MBH to stimulate LHRH release is not yet elucidated. Arcuate neurons bearing Ob receptors may project to the ME to the tanycyte/portal capillary junction. Leptin would either combine with its receptors on the terminals that transmit information to the cell bodies in the AN or diffuse to the AN to combine with its receptors on the perikarya of AN neurons. Because leptin decreases NPY mRNA and presumably NPY biosynthesis in NPY neurons in the AN, we postulate that leptin causes a decrease in NPY release. Because NPY inhibited LH release in intact and castrated male rats (55), we hypothesize that NPY decreases the release of LHRH by inhibiting the noradrenergic neurons that mediate pulsatile release of LHRH. Therefore, when the release of NPY is inhibited by leptin, noradrener-

gic impulses are generated that act on an $\alpha_1$ receptor on the NOergic neurons, causing the release of NO that diffuses to the LHRH terminals and activates LHRH release by activating guanylate cyclase and cyclooxygenase$_1$ as shown in our prior experiments, reviewed previously. Leptin acts to activate NOS as indicated because its release of LHRH is blocked by inhibition of NOS (56). The LHRH enters the portal vessels and is carried to the anterior pituitary gland, where it acts to stimulate FSH and particularly LH release by combining with its receptors on the gonadotropes. The release of LH and to a lesser extent FSH is further increased by the direct action of leptin on its receptors in the pituitary gland (56,58,62).

Actively or permissively leptin may be a critical factor in the induction of puberty as the animal nears the so-called critical weight. Either metabolic signals reaching the adipocytes or signals related to their content of fat causes the release of leptin, which increases LHRH and gonadotropin release, thereby facilitating puberty. In the male, the system would work similarly; however, there is no preovulatory LH surge brought about by the positive feedback of estradiol. Sensitivity to leptin is undoubtedly under steroid control, and we are actively working to elucidate this problem.

During fasting, the leptin signal is removed and LH pulsatility and reproductive function decline quite rapidly. In women with anorexia nervosa, this causes a reversion to the prepubertal state, which can be reversed by feeding. Thus, leptin would have a powerful influence on reproduction throughout the reproductive lifespan of the individual. The consequences of gonadotropin secretion of overproduction of leptin, as already demonstrated in human obesity, are not clear. There are often reproductive abnormalities in this circumstance and whether they are due to excess leptin production or other factors remains to be determined. In conclusion, it is now clear that leptin plays an important role in control of reproduction by actions on the hypothalamus and pituitary.

## References

1. McCann SM, Taleisnik S, Friedman KM. LH-releasing activity in hypothalamus extracts. Proc Soc Exp Biol Med 1960;104:432.
2. McCann SM, Ojeda SR. The anterior pituitary and hypothalamus. In: Griffin J, Ojeda SR, editors. Textbook of endocrine physiology. 3rd ed. New York: Oxford University Press;1996. p. 101.
3. Reichlin S. Neuroendocrinology. In: Wilson JD, Foster DW, editors. Williams' textbook of endocrinology. Philadelphia, PA, USA: WB Saunders;1992; p. 135.
4. McCann SM, Marubayashi U, Sun H-Q, Yu WH. Control of follicle stimulating hormone and luteinizing hormone release by hypothalamic peptides. In: Chrousos GP, Tolis G, editors. Intraovarian regulators and polycystic ovarian syndrome: recent progress on clinical and therapeutic aspects. Ann N Y Acad Sci 1993;687:55.
5. Culler MD, Negro-Vilar A. Pulsatile follicle-stimulating hormone secretion is independent of luteinizing hormone-releasing hormone (LHRH): pulsatile replacement of LHRH bioactivity in LHRH-immunoneutralized rats. Endocrinology 1987;120:2011.
6. Dees WL, Rettori V. Kozlowski JG, McCann SM. Ethanol and the

pulsatile release of luteinizing hormone, follicle stimulating hormone and prolactin in ovariectomized rats. Alcohol 1985;2:641.

7. Rettori V, Gimeno MF, Karara A, Gonzales MC, McCann SM. Interleukin lα inhibits prostaglandin $E_2$ release to suppress pulsatile release of luteinizing hormone but not follicle-stimulating hormone. Proc Natl Acad Sci USA 1991;88:2763.

8. McCann SM, Krulich L. Role of neurotransmitters in control of anterior pituitary hormone release. Endocrinology. 2nd ed. Philadelphia, PA, USA: WB Saunders;1989. p. 117.

9. Lumpkin MD, McDonald JK, Samson WK, McCann SM. Destruction of the dorsal anterior hypothalamic region suppresses pulsatile release of follicle stimulating hormone but not luteinizing hormone. Neuroendocrinology 1989;50:229.

10. Marubayashi U, Yu WH, McCann SM. Median eminence lesions reveal separate hypothalamic control of pulsatile follicle-stimulating hormone and luteinizing hormone release. Proc Soc Exp Biol Med 1999;220:139.

11. Igarashi M, McCann SM. A hypothalamic follicle stimulating hormone releasing factor. Endocrinology 1964;74:446.

12. Dhariwal APS, Nallar R, Batt M, McCann SM. Separation of FSH-releasing factor from LH-releasing factor. Endocrinology 1965;76:290.

13. Dhariwal APS, Watanabe S, Antunes-Rodrigues J, McCann SM. Chromatographic behavior of follicle stimulating hormone-releasing factor on Sephadex and carboxy methyl cellulose. Neuroendocrinology 1967;2:294.

14. Schally AV, Saito T, Arimura A, Muller EE, Bowers CY, White WF. Purification of follicle-stimulating hormone-releasing factor (FSH-RF) from bovine hypothalamus. Endocrinology 1966;79:1087.

15. Lumpkin MD, Moltz JH, Yu WH, Samson WK, McCann SM. Purification of FSH-releasing factor: its dissimilarity from LHRH of mammalian, avian, and piscian origin. Brain Res Bull 1987;18:175.

16. Mizunuma H, Samson WK, Lumpkin MD, Moltz JH, Fawcett CP, McCann SM. Purification of a bioactive FSH-releasing factor (FSHRF). Brain Res Bull 1983;10:623.

17. Yu WH, Karanth S, Sower SA, Parlow AF, McCann SM. The similarity of FSH-releasing factor to lamprey gonadotropin-releasing hormone III (lGnRH-III). Proc Soc Exp Biol Med 2000;224:87.

18. Yu WH, Millar RP, Milton SCF, Del Milton RC, McCann SM. Selective FSH-releasing activity of [D-Trp9] GAP 1-13: comparison with gonadotropin-releasing abilities of analogs of GAP and natural LHRHs. Brain Res Bull 1990;25:867.

19. Yu WH, Karanth S, Walczewska A, Sower SA, McCann SM. A hypothalamic follicle-stimulating hormone-releasing decapeptide in the rat. Proc Natl Acad Sci USA 1997;94:9499.

20. Dees WL, Hiney JK, Sower SA, Yu WH, McCann SM. Localization of immunoreactive lamprey gonadotropin-releasing hormone in the rat brain. Peptides 1999;20:1503.

21. Lumpkin MD, McCann SM. Effect of destruction of the dorsal anterior hypothalamus on selective follicle stimulating hormone secretion. Endocrinology 1984;115:2473.

22. Lumpkin MD, McDonald JK, Samson WK, McCann SM. Destruction of the dorsal anterior hypothalamic region suppresses pulsatile release of follicle stimulating hormone but not luteinizing hormone. Neuroendocrinology 1989;50:220.

23. Ojeda SR, Jameson HE, McCann SM. Hypothalamic areas involved in prostaglandin (PG)-induced gonadotropin release. II. Effect of PGE2 and PGF2 implants on follicle stimulating hormone release. Endocrinology 1997;100:1595.

24. Yu WH, Karanth S, Mastronardi CA, Sealfon S, Dees L, McCann SM. Follicle stimulating hormone-releasing factor acts on its putative receptor via nitric oxide to specifically release follicle stimulating hormone. 81st Annual Meeting of the Endocrine Society. San Diego, CA, USA. p. 2, 284. June 12–15, 1999.

25. Childs GV, Miller BT, Chico DE, Unabia GC, Yu WH, McCann SM. Preferential expression of receptors for lamprey gonadotropin releasing hormone-III (GnRH-III) by FSH cells: support for its function as an FSH-RF (Abstract #P3-268). The Endocrine Society's 83rd Annual Meeting. Denver, CO, USA. June 20–23, 2001. p. 506.

26. Wang L, Bogerd J, Choi HS, Seong JY, Soh JM, Chun SY, Blomenrohr M, Troskie BE, Millar RP, Yu WH, McCann SM, Kwon HB. Three distinct types of GnRH receptor characterized in the bullfrog. Proc Natl Acad Sci USA 2001;98:361.

27. Neil JD, Duck LW, Sellers JC, Musgrove LC. A gonadotropin-releasing hormone (GnRH) receptor specific for GnRH II in primates. Biochem Biophys Res Commun 2001;282:1012.

28. Lescheid DW, Terasawa EI, Abler LA, Urbanski HF, Warby CM, Millar RP, Sherwood NM. A second form of gonadotropin-releasing hormone (GnRH) with characteristics of chicken GnRH-II is present in the primate brain. Endocrinology 1997;138:5618.

29. Wakabayashi K, Kamberi IA, McCann SM. *In vitro* responses of the rat pituitary to gonadotrophin-releasing factors and to ions. Endocrinology 1969;85:1046.

30. Stojilkovic SS. Calcium signaling systems. In: Conn MP, Goodman HM, editors. Handbook of physiology. Section 7. The endocrine system. Vol. I: Cellular endocrinology. New York: Oxford Press;1998. p. 177.

31. Nakano H, Fawcett CP, Kimura F, McCann SM. Evidence for the involvement of guanosine $3',5'$-cyclic monophosphate in the regulation of gonadotropin release. Endocrinology 1978;103:1527.

32. Naor Z, Fawcett CP, McCann SM. The involvement of cGMP in LHRH stimulated gonadotropin release. Am J Physiol 1978;235:586.

33. Snyder G, Naor Z, Fawcett CP, McCann SM. Gonadotropin release and cyclic nucleotides: evidence for LHRH-induced elevation of cyclic GMP levels in gonadotropes. Endocrinology 1980;107:1627.

34. Yu WH, Karanth S, Mastronardi C, Sealfon S, Dees L, McCann SM. Follicle stimulating hormone-releasing factor acts on its putative receptor via nitric oxide to specifically release follicle stimulating hormone. 81st Annual Meeting of the Endocrine Society. San Diego, CA, USA. June 12–15, 1999. p. 2, 284.

35. Dees WL. Dearth RK, Hooper RN, Brinsko SP, Romano JE, Rahe CH, Yu WH, McCann SM. Lamprey gonadotropin-releasing hormone-III selectively releases follicle stimulating hormone in the bovine. Domes Anim Endocrinol 2001;20:279.

36. McCann SM, Rettori V. The role of nitric oxide in reproduction. Soc Exp Biol Med 1996;211:7.

37. Rettori V, Belova N, Dees WL, Nyberg CL, Gimeno M, McCann SM. Role of nitric oxide in the control of luteinizing hormone-releasing hormone release *in vivo* and *in vitro*. Soc Exp Biol Med 1996;211:7.

38. Mani SK. Allen JMC, Rettori V, O'Malley BW, Clark JH, McCann SM. Nitric oxide mediates sexual behavior in female rats by stimulating LHRH release. Proc Natl Acad Sci USA 1994;91:6468.

39. Rettori V, Gimeno M, Lyson K, McCann SM. Nitric oxide mediates norepinephrine-induced prostaglandin $E_2$ release from the hypothalamus. Proc Natl Acad Sci USA 1992;89:11543.

40. Canteros G, Rettori V, Franchi A, Genaro A, Cebral E, Saletti A, Gimeno M, McCann SM. Ethanol inhibits luteinizing hormone-releasing hormone (LHRH) secretion by blocking the response of LHRH neuronal terminals to nitric oxide. Proc Natl Acad Sci USA 1995;92:3416.

41. Bredt DS, Snyder SH. Nitric oxide mediates glutamate-linked enhancement of cGMP levels in the cerebellum. Proc Natl Acad Sci USA 1989;86:9030.

42. Canteros G, Rettori V, Genaro A, Suburo A, Gimeno M, McCann SM. Nitric oxide synthase (NOS) content of hypothalamic explants: increase by norepinephrine and inactivated by NO and cyclic GMP. Proc Natl Acad Sci USA 1996;93:4246.

43. Kamat A, Yu WH, Rettori V, McCann SM. Glutamic acid stimulated luteinizing-hormone releasing hormone release is mediated by alpha adrenergic stimulation of nitric oxide release. Brain Res Bull 1995; 37:233.

44. Rettori V, Canteros G, Wrens R, Gimeno M, McCann SM. Oxytocin stimulates the release of luteinizing hormone-releasing hormone from medial basal hypothalamic explants by releasing nitric oxide. Proc Natl Acad Sci USA 1997;94:2741.

45. Seilicovich A, Duvilanski BH, Pisera D, Thies S, Gimeno M, Rettori V, McCann SM. Nitric oxide inhibits hypothalamic luteinizing hor-

mone-releasing hormone release by releasing γ-aminobutyric acid. Proc Natl Acad Sci USA 1995;92:3421.

46. Seilicovich A, Lasaga M. Befurno M, Duvilanski BH, de C Dias M, Rettori V, McCann SM. Nitric oxide inhibits the release of norepinephrine and dopamine from the medial basal hypothalamus of the rat. Proc Natl Acad Sci USA 1995;92:11299.

47. Rettori V, Belova N, Kamat A, Lyson K, McCann SM. Blockade by IL-I-alpha of the nitrocoxidergic control of luteinizing hormone-releasing hormone release *in vivo* and *in vitro*. Neuroimmunomodulation 1994;1:86.

48. Kimura M. Yu WH, Rettori V, McCann SM. Granulocyte-macrophage colony stimulating factor suppresses LHRH release by inhibition of nitric oxide synthase and stimulation of gamma-aminobutyric acid release. Neuroimmunomodulation 1997;4:237.

49. Swerdloff R, Batt R, Bray G. Reproductive hormonal function in the genetically obese (ob/ob) mouse. Endocrinology 1976;98:1359.

50. Swerdloff R, Peterson M, Vera A, Batt R. Heber D, Bray G. The hypothalamic-pituitary axis in genetically obese (ob/ob) mice: response to luteinizing hormone-releasing hormone. Endocrinology 1978;103:542.

51. Chehab FF, Lim ME, Ronghua L. Correction of the sterility defect in homozygous obese female mice by treatment with the human recombinant leptin. Nat Genet 1996;12:318.

52. Barash IA, Cheung CC, Weigle DS, Ren H, Kabigting EB, Kuijper JL, Clifton DK, Steiner RA. Leptin is a metabolic signal to the reproductive system. Endocrinology 1996;1(37):3144.

53. Frisch RE, McArthur JW. Menstrual cycles: fatness as a determinant of minimum weight for height necessary for their maintenance or onset. Science 1974;185:949.

54. Ronnekleiv OK, Ojeda SR, McCann SM. Undernutrition, puberty and the development of estrogen positive feedback in the female rat. Biol Reprod 1978;19:414.

55. Dees WL, Dearth RK, Hooper RN, Brineko SP, Roman JE, Rahe H, Yu WH, McCann SM. Lamprey GNRHIII selectivity releases FSH in the bovine. Domest Anim Endocrinol 2001;20:279.

56. Yu WH, Kimura M, Waiczewska A, Karanth S, McCann SM. Role of leptin in hypothalamic-pituitary function. Proc Natl Acad Sci USA 1997;94:1023.

57. Walczewska A, Yu WH, Karanth S, McCann SM. Estrogen and leptin have differential effects on FSH and LH release in female rats. Proc Soc Exp Biol Med 1999;222:70.

58. Cioffi J, Shafer A, Zupancic T, Smith-Gbur J, Mikhail A, Platika D, Snodgrass H. Novel B219/ob receptor isoforms: possible role of leptin in hematopoiesis and reproduction. Nat Med 1996;2:585.

59. Schwartz MW, Seeley RJ, Campfield LA, Burn P, Baskin DG. Identification of targets of leptin action in rat hypothalamus. J Clin Invest 1996;98:1101.

60. Banks WA. Kastin AJ, Huang W, Jaspan JB, Maness LM. Leptin enters the brain by a saturable system independent of insulin. Peptides 1996;17:305.

61. Vaisse C, Halaas JL, Horvath CM, Darnell JE Jr, Stoffel M, Friedman JM. Leptin activation of stat3 in the hypothalamus of wildtype and Ob/Ob mice but not Db/Db mice. Nat Genet 1996;14:95.

62. Naivar JS, Dyer CJ, Matteri RL, Keisler DH. Expression of leptin and its receptor in sheep tissues. [Abstract]. Proc Soc Study Reprod 1996; 391:154.