GnRH PULSES—THE REGULATORS OF HUMAN REPRODUCTION

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INTRODUCTION

Gonadal function in mammals is controlled by the dual action of the pituitary gonadotropins luteinizing hormone (LH) and follicle-stimulating hormone (FSH). Both synthesis and secretion of LH and FSH are regulated by gonadotropin releasing hormone (GnRH). GnRH is secreted by the hypothalamus in a pulsatile manner (1, 2) and this intermittent pattern of GnRH stimulation is essential for the maintenance of gonadotropin synthesis and secretion (3, 4). LH and FSH consist of a common alpha and different beta subunits (5) which are encoded by three separate genes (6). Both hormones are secreted by the same pituitary gonadotrope cells and the differential regulation of LH and FSH secretion appears to be effected by variations in the pattern of the GnRH pulse stimulus, together with the direct effects of gonadal steroids and peptides on the gonadotrope cell.

The pattern of pulsatile GnRH secretion varies during reproductive life (7–13) and is regulated in part by the feedback actions of gonadal steroids. Testosterone in men, and estradiol and progesterone together in women slow the frequency of GnRH secretion by an action which involves hypothalamic opioid peptides. In children, GnRH secretion occurs at a low amplitude and slow frequency and both amplitude and frequency increase during pubertal maturation. In adult men, GnRH secretion appears to be invarying with pulses of GnRH occurring approximately every two hours. In women, the pattern of GnRH pulses changes during the ovulatory menstrual cycle, with pulse frequency gradually increasing during the follicular phase and amplitude also being increased during the mid-cycle ovulatory LH and FSH surge. Following

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ovulation, pulse frequency slows due to the feedback effect of estradiol and progesterone from the corpus luteum.

Thus, the overall regulation of mammalian reproduction results from a complex feedback system involving regulation of the pattern of hypothalamic GnRH secretion by gonadal hormones. Gonadal steroids can also modify gonadotrope responses to GnRH. Testosterone generally reduces LH responses and estradiol has a biphasic effect with initial inhibition, and subsequent enhancement of LH responses to GnRH. Gonadal peptides such as inhibin, activin and follistatin can exert direct actions on the gonadotrope to reduce (inhibin) or enhance FSH secretion. The interplay of these feedback mechanisms at both the hypothalamus and pituitary level effects differential synthesis and secretion of the two gonadotropins. This in turn allows orderly delivery of FSH and LH to effect follicular development and steroid secretion in the ovary and spermatogenesis and steroid secretion in the testes.

In this review we examine the role of the ability to change GnRH pulsatile secretion in regulating gonadotropin synthesis and secretion and subsequent gonadal responsiveness. First, we discuss GnRH regulation of gonadotropin subunit gene expression. Subsequently, we explore the changes in pulsatile GnRH secretion which occur during sexual maturation and during normal ovulatory cycles. We also examine abnormalities of GnRH secretion which are seen in anovulatory conditions, such as hypothalamic amenorrhea, hyperprolactinemia or the polycystic ovary syndrome.

REGULATION OF GONADOTROPIN SUBUNIT GENE EXPRESSION

Gonadotropin subunit mRNA expression during the rat estrous cycle

In cycling female rats, plasma LH and FSH are low except during the preovulatory surge on the afternoon of proestrus (14). In contrast, changes in gonadotropin mRNA concentrations occur on different days of the cycle (15, 16). On the morning of metestrus only FSH beta mRNA is increased. On the following day (diestrus) both alpha and LH beta mRNAs are increased while FSH beta remains unchanged. On proestrus, LH beta mRNA increases prior to the preovulatory rise in LH and FSH beta mRNA is also increased beginning at the time of the rise in serum FSH. Alpha mRNA concentrations were unchanged during the gonadotropin surges. These observations of steady state mRNA concentration are supported by measurements of subunit gene transcription rates. These studies showed that alpha subunit transcription did not change throughout the cycle, while LH beta and FSH beta transcription rates were highest on the afternoons of proestrus and estrus respectively (17).

Thus, during the estrous cycle, the subunit genes are expressed both coordinately and differentially, but the exact mechanisms effecting these changes remain uncertain. The pattern of pulsatile GnRH secretion changes during the cycle, with both amplitude and frequency being increased on proestrus (18–19). The amplitude of GnRH pulses is known to regulate mRNA expression in female rates (20) and secretion of LH and FSH can be altered by changes in circulating estradiol (E_2) and progesterone (P) during the cycle. Thus, changes in GnRH pulse pattern, perhaps modified by the actions of E_2 and P on the gonadotrope may act to differentially regulate subunit gene expression. In particular, inhibin can inhibit FSH release and reduce FSH beta mRNA concentrations in vitro and in vivo (21–23) and plasma inhibin levels show a generally inverse relationship to plasma FSH and FSH beta subunit mRNA concentrations (24).

The role of gonadal steroids and peptides in regulating gonadotropin subunit gene expression

Following gonadectomy, both serum gonadotropins and all three subunits mRNAs increase, but the timing and the magnitude of the changes differ, both between subunits and between the sexes (25–28). In females, serum LH, alpha and LH beta mRNAs do not increase until several days after ovariectomy, while FSH beta mRNA rises within a few hours. In males, both alpha and LH beta mRNA increase within 24 hours of castration and continue to increase over the subsequent four weeks. FSH beta mRNA also increases, but plateaus after seven days (29).

In female rats, replacement with estradiol at the time of ovariectomy prevents the increase in LH beta mRNA. The rise in alpha mRNA persists however, and the increase in FSH beta mRNA is only partially suppressed. Estradiol and progesterone together produce similar effects, which are similar to the changes following administration of a GnRH antagonist to ovariectomized rats (30). Thus, the increase in FSH beta mRNA after ovariectomy occurs in the absence of GnRH stimulation of the gonadotrope. Recent data have shown that the rapid increase in FSH beta mRNA reflects the loss of ovarian inhibin, by actions which appear to involve stabilization of the FSH beta message (31). In male rats, replacement of testosterone at castration prevents the increase in all three subunit mRNAs, in similar manner to the effects of a GnRH antagonist (32). This suggests that the increase in subunit mRNAs reflects increasing GnRH secretion, though testosterone may also exert direct effects on FSH beta mRNA stability (33). Administration of testosterone to castrate males treated with a GnRH antagonist increased FSH beta mRNA two-fold and prolonged the disappearance time of FSH beta mRNA from cytoplasm by a factor of two (34).

These data suggest that estradiol and progesterone in females and testosterone in males act to reduce the increase in GnRH secretion which occurs after gonadectomy (35, 36). Both E_2 and P in females and testosterone in males also appear to selectively increase FSH beta mRNA concentrations, which does not appear to involve a change in transcription rate and suggests an action effected by stabilization of the FSH beta mRNA message (34, 37). Inhibin appears to exert an opposite effect, rapidly reducing FSH beta mRNA concentrations (38, 39). Present data are incomplete, but this action appears to be mediated by reducing the stability of FSH beta mRNA. Activin and follistatin are peptides which were first identified in the gonads but which are also present in the pituitary. Both peptides increase FSH beta mRNA concentrations by actions which appear to involve stabilization of the FSH beta mRNA (38).

Regulation of subunit gene expression by GnRH pulses

As noted above, a pulsatile GnRH stimulus is essential to maintain gonadotropin secretion and LH and FSH secretion falls in the presence of a continuous GnRH stimulus—desensitization of the gonadotrope (3, 40). After desensitization, alpha mRNA is increased while LH beta mRNA is decreased (41, 42), and a pulsatile stimulus is known to be required to stimulate beta subunit transcription (43). GnRH antagonists reduce both transcription and steady state concentrations of subunit mRNAs (44), which emphasizes the role of GnRH and also the importance of an intermittent GnRH signal.

We have investigated the role of GnRH pulse pattern in male rats by administering exogenous GnRH to a relatively GnRH deficient rat model. Castrate male rats received testosterone replacement by implants, which reduces endogenous GnRH secretion to a low or undetectable level (40, 45). Similar data are lacking in females due to the absence of an equivalent female model and hence the data below were obtained during studies in males.

Effects of GnRH pulse amplitude

In castrate rats, GnRH pulses occur every 30 minutes and we have administered different amplitudes (1-250 ng/pulse) of GnRH at a frequency of one pulse every 30 minutes. All amplitudes of GnRH pulses increased both alpha and FSH beta mRNAs. In contrast, both LH beta mRNA and LH secretion showed an amplitude optimum, with 25 ng pulses producing the highest response. Thus, the changes in the amplitude of the GnRH pulse can effect differential expression of the three subunit mRNAs (46–48).

These studies also indicated differences in the time course of subunit mRNA responses to GnRH pulses. FSH beta mRNA increased within 4–12 hours, alpha mRNA by 12 hours, while LH beta mRNA did not show a measurable increase until after 24 hours of GnRH pulses.

Effects of GnRH pulse frequency

The effects of a constant pulse amplitude (25 ng) given at different pulse intervals (8–480 minutes) were also examined in the same male rat model. Pulses faster than occur in normal physiology (every 8 minutes), increased alpha mRNA and to a lesser degree LH beta mRNA. Pulses given every thirty minutes (the frequency present in castrate rats) increased all three subunit mRNA concentrations, whereas slower frequency pulses only maintained expression of FSH beta mRNA (49–51). These effects appear to be exerted at the level of gene transcription (52), with fast frequency pulses increasing alpha transcription rates, slow frequency pulses increasing FSH beta transcription, while LH beta transcription rate was only increased by 30 minute pulses.

These data suggest that changes in both the amplitude and frequency of the GnRH pulses stimulus can effect differential expression of the gonadotropin subunit genes which appear to be exerted at the level of transcription. The data also suggest that the observed physiologic changes in pulsatile GnRH secretion seen during pubertal maturation and during ovulatory cycles may be one of the mechanisms involved in effecting differential synthesis and secretion of the two pituitary gonadotropic hormones (53).

PULSATILE GnRH SECRETION IN HUMAN SUBJECTS

Pubertal maturation

In prepubertal girls and boys, LH (by inference GnRH) pulse amplitude is very low and observed pulses occur at a slow frequency of approximately every 3–4 hours (9, 10, 54–56). A minor augmentation of pulse amplitude is seen with the onset of sleep before puberty, and a marked amplification of both the amplitude and frequency of pulsatile GnRH secretion heralds the onset of pubertal maturation. Initially, GnRH pulses increase with the onset of sleep and frequency increases to approximately one pulse per hour. Over time, this enhanced GnRH pulsatility continues for a longer duration and in adults persists throughout 24 hours. In boys, the consequent increase in FSH and LH stimulates testosterone secretion. This exerts an inhibitory effect on pulse frequency so that in adult men, pulses occur approximately every two hours. In girls, fewer data are available, but it is suggested that the increase in estradiol alone does not

slow the frequency of GnRH pulses. This continuing GnRH stimulation results in gonadotrope stimulation of the ovary and waves of incomplete follicular development. Estradiol also augments LH responsiveness to GnRH and may result in LH surges during anovulatory cycles. Subsequent luteinization of maturing follicles and secretion of progesterone may effect the slowing of GnRH secretion which is seen during established ovulatory cycles.

During the process of pubertal maturation, the patterns of gonadotropin responsiveness to exogenous GnRH also change. Before puberty, particularly in girls, the predominant response is one of FSH secretion, while after puberty LH responses exceed those of FSH. This supports the suggestion that the development of a continuing rapid frequency GnRH pulse stimulus favors expression of the LH beta gene and LH secretion.

GnRH secretion during ovulatory menstrual cycles

During the follicular phase of an ovulatory cycle, the initial monotropic increase of plasma FSH declines and plasma LH gradually increases to a peak at the mid-cycle LH surge. The initial increase in FSH stimulates follicular recruitment and maturation and the consequent secretion of estradiol selectively inhibits FSH release and may maintain (or stimulate) a rapid GnRH pulse frequency during the late follicular phase (7, 11, 13, 57–58). The persistent rapid GnRH stimulus increases plasma LH, which further stimulates estradiol secretion and the positive feedback of estradiol enhances LH responsiveness to produce the mid-cycle LH surge (59–61). GnRH pulse frequency increases from one pulse every 90–100 minutes in the early follicular phase to a frequency of approximately one pulse per hour during the late follicular phase and during the ovulatory surge. Studies in primates have shown continuing pulsatile LH secretion during the surge with LH pulse amplitude increasing on the ascending, and decreasing on the descending portion of the LH surge. Direct measurements of GnRH in hypothalamic-portal blood of sheep after administration of estradiol has shown an increase in GnRH pulse frequency and amplitude (62). During the LH surge itself GnRH levels appear to be consistently elevated and remain elevated as plasma LH declines (63). This suggests that the frequency of GnRH pulse secretion has become very rapid, or is even continuous, which results in desensitization of LH secretion, perhaps the mechanism of termination of the LH surge. After ovulation, luteinization of the ruptured follicle results in progesterone secretion which together with estradiol acts at the hypothalamic level to increase opioid activity, which in turn reduces the frequency of GnRH pulses. In the first few days after ovulation pulses occur every

60–90 minutes, but progressively slow to a frequency of every 3–5 hours by the mid-luteal phase of the cycle. Evidence that the slowing of GnRH secretion by estradiol and progesterone is due to enhanced endogenous opioid activity has been shown in several studies. Administration of progesterone during the follicular phase slows GnRH pulses (64), and blockade of the opiate receptor by naloxone increases GnRH pulse frequency during the mid-luteal phase. In line with our earlier studies in rats, the slow luteal frequency of GnRH pulses would favor FSH synthesis, but release of FSH is inhibited by the combined suppressive effects of estradiol and inhibin for the corpus luteum. Thus, the pituitary content of FSH would be expected to increase. In contrast, slow irregular GnRH pulses would not be optimal for LH synthesis, but as LH release occurs in response to the irregular GnRH stimulus, pituitary LH stores would be expected to decline. With the demise of the corpus luteum, serum estradiol, progesterone and inhibin levels fall in plasma. GnRH pulse frequency increases and plasma FSH rises due to removal of the inhibitory effects of estradiol and inhibin. This leads to the initial monotropic increase of FSH, which stimulates follicular maturation in the next cycle.

These observations have been confirmed in several laboratories and together suggest a consistent pattern of change in pulsatile GnRH secretion during ovulatory menstrual cycles. The data also suggest, together with studies in rodents, that the altered GnRH stimulus is important in differential synthesis and secretion of FSH—which is essential for orderly follicular maturation and ovulation (53, 65). However, it should be noted that ovulation can be induced in GnRH deficient humans or primates by administration of fixed doses of GnRH at fixed frequencies (66–68). In a majority of these studies a supraphysiologic dose of exogenous GnRH was used, which may override the need to change the frequency of the GnRH stimulus. Additionally, most studies involve one ovulatory cycle and it is uncertain whether the ability to alter GnRH frequency is important in maintaining ovulatory cycles over prolonged periods of time. It may be postulated that each monotropic increase in FSH at the beginning of the follicular phase initiates maturation of follicles destined to ovulate in future cycles, in addition to stimulating maturation of follicles involved in the present cycle. Some data to support this view are found in studies which have shown that administration of rapid pulsatile GnRH during the luteal phase can result in deficient follicular development and impaired corpus luteum function in subsequent cycles (69, 70).

The observed changes in GnRH stimulation of the pituitary during the follicular and luteal phases of an ovulatory cycle may involve similar mechanisms to those operative during puberty. During the follicular phase increasing GnRH pulse frequency and amplitude and the reversal

in plasma gonadotropins, declining FSH and increasing LH, are similar to the changes observed during pubertal maturation (53).

PULSATILE GnRH SECRETION IN ANOVULATORY CONDITIONS

Recent studies have shown that several types of reproductive dysfunction resulting in anovulation and/or amenorrhea appear to be associated with abnormalities of sequential changes in pulsatile GnRH secretion. These suggest that the abnormal GnRH stimulus, and particularly an inability to sequentially change GnRH pulse frequency may be the underlying abnormality in anovulation in these women.

Hypothalamic amenorrhea

Hypothalamic amenorrhea (HA) is a relatively common disorder which is a diagnosis made only after exclusion of pituitary and ovarian abnormalities. The amenorrhea is commonly preceded by marked weight loss, strenuous exercise such as competitive running or gymnastics, psychological stress or on occasion the use of oral contraceptives (71–72). The disorder is reversible and in many women ovulatory menses resume within one year of removal of the antecedent conditions. Basal measurements of plasma LH, FSH, prolactin and estradiol show that these are usually normal or slightly low, and responsiveness to exogenous GnRH is maintained. Studies in several laboratories have shown that the frequency of GnRH pulse secretion is often markedly reduced in a majority of women with HA (13, 57, 73). GnRH pulses occur at a slow frequency (every 3–4 hours) and irregular amplitude, similar to the patterns observed during the luteal phase of a normal cycle. This suggests that similar mechanisms may be involved in reducing endogenous GnRH secretion in the two situations. This suggestion is confirmed in some patients by restoration of pulsatile GnRH secretion within hours after administration of the opiate receptor blocker naloxone (74–76). In addition, some reports have indicated that long term administration of the orally active opiate receptor blocker naltrexone can result in ovulation when given over periods of several weeks (77). These data indicate that in a majority of women with HA, the anovulation appears to reflect a persistent slow frequency GnRH stimulus which is inadequate to increase LH synthesis and secretion to the level required for an ovulatory LH surge. It should be noted, however, that a significant number of women with HA are unresponsive to naloxone (76) and the mechanisms involved in the disordered GnRH secretion in this group remain unclear.

Hyperprolactinemia

Anovulation and amenorrhea are commonly associated with an elevation of serum prolactin in women. In situations where prolactin is elevated as a result of a prolactinoma, or where the hyperprolactinemia reflects the use of medications which reduce hypothalamic dopamine secretion or action, the effects on the reproductive axis appear to be similar. Studies of pulsatile LH secretion have revealed slow, irregular patterns suggesting inhibition of hypothalamic GnRH secretion. Administration of bromocriptine, a dopamine agonist, reduces prolactin and restores pulsatile LH secretion to normal (78, 79), which is followed by resumption of ovulatory cycles. Of interest, some studies suggest that prolactin reduces GnRH secretion by an action which also involves excess hypothalamic opioid activity. In hyperprolactinemic women, administration of naloxone did not lower serum prolactin, but restored a normal frequency of pulsatile GnRH secretion (80, 81). This suggests that the elevated prolactin increases hypothalamic opioid activity which in turn reduces GnRH pulse frequency and prevents the normal increase in pulse frequency which occurs during the follicular phase of ovulatory cycles.

Polycystic ovarian syndrome

Polycystic ovarian syndrome (PCO) is a relatively common disorder which is associated with anovulation, hirsutism, multiple cysts in the ovaries, and obesity. The clinical syndrome may reflect several underlying causes, but the excess androgen production is essentially of ovarian origin, and in a majority of women appears to reflect increased LH secretion (82–84). Administration of a GnRH antagonist rapidly reduces LH and androgen secretion, indicating a role for LH stimulation of ovarian steroidgenesis in the excess androgen secretion. Recent studies have shown that both the frequency and amplitude of LH pulses are increased in patients with PCO (85, 86). Pulses of LH are secreted persistently at a frequency of approximately one pulse per hour—similar to that present during the late follicular phase of an ovulatory cycle. In such circumstances of persistent pulsatile GnRH secretion, FSH synthesis and secretion will be expected to decline, but LH synthesis and secretion would increase, with resultant enhanced androgen production by the ovaries. These latter changes summarize the observed hormonal abnormalities in women with PCO.

In women with PCO where LH levels are elevated, we have recently proposed that the underlying abnormality reflects a reduced sensitivity of the GnRH pulse generator to the actions of estradiol and progesterone in slowing GnRH secretion. The disorder commonly begins around

menarche and if a pubertal girl was relatively insensitive to the effects of low levels of progesterone (present in the anovulatory cycles seen in the first few months after menarche), slowing of GnRH pulsatile secretion would not occur and the cyclic changes observed in ovulatory cycles will be absent. Persistent GnRH pulses would favor LH and not FSH synthesis and secretion and subsequent follicular development would be impaired. In the absence of follicular luteinization and progesterone secretion, a rapid GnRH pulse frequency might be maintained, leading to persistent anovulation and continuing deficiency of progesterone—removing the signal to slow GnRH pulses.

We have examined this hypothesis by administering mid-luteal concentrations of estradiol and progesterone to women with PCO (87). Administration of E_2 and P for 20 days resulted first in a decrease in GnRH pulse frequency, and a later reduction in LH pulse amplitude. After discontinuing the E_2 and P, GnRH pulse frequency increased with a selective increase in plasma FSH, LH levels remaining low during the first week after steroids have been discontinued. As a consequence LH/FSH ratios returned to unity and administration of exogenous GnRH at this time showed that LH responses were impaired compared to those of FSH. Thus reducing the frequency of the GnRH stimulus for 2–3 weeks may have reduced LH synthesis. The selective increase in FSH after steroid withdrawal suggests that FSH synthesis was maintained, allowing selective FSH secretion in response to the increase in GnRH pulse frequency. The FSH secreted in these circumstances appears to be bioactive, resulting in follicular maturation in all women and ovulation in some.

These preliminary data are supportive of the concept that a majority of women with PCO reflect an abnormality of GnRH secretion from the hypothalamus. However, further studies are required to determine if slowing of the GnRH stimulus will allow normalization of the intraovarian milieu, and if selective FSH release after steroid withdrawal will consistently stimulate ovarian follicular maturation and ovulation.

SUMMARY

The data reviewed in this chapter provide evidence that the pattern of GnRH secretion appears to be an important factor in regulating gonadotropin subunit gene expression, gonadotropin synthesis and hormone secretion. The data on gonadotropin synthesis were obtained in rodents and hence, must be interpreted with caution when applied to primates. Despite this reservation, the data suggest a similarity of regulatory mechanisms in mammalian species. The data also provide an explanation for the mechanisms whereby a single gonadotropin-releasing hormone

can differentially regulate the three gonadotropin genes and allow differential hormone secretion.

In overall agreement with this view, the observations during pubertal maturation reveal increasing GnRH pulsatile secretion during puberty with an evolution from predominant FSH to a predominant LH secretion by the gonadotropes. In males, the patterns of GnRH secretion appear to be fairly consistent throughout adult life, but in women cyclic changes occur which perhaps are important in maintaining cyclic ovulation. It is proposed that once pubertal maturation has been established, GnRH is secreted at a relatively fast frequency (one pulse per hour), and an essential feature of repeated ovulatory cycles is the slowing of this GnRH stimulus during the luteal phase:—to allow subsequent preferential FSH release. This slowing of GnRH secretion appears to be effected by estradiol and progesterone acting to enhance hypothalamic opioid activity. Similar mechanisms involving increased opioid tone appear to be causally related to the reduced frequency and irregular GnRH stimulus seen in hypothalamic amenorrhea and hyperprolactinemia. In contrast, some forms of polycystic ovarian disease may reflect abnormalities of the estradiol-progesterone/opioid/GnRH neuron feedback mechanisms, with failure to establish slowing in the peripubertal anovulatory cycles. The resulting persistent GnRH stimulus increases LH with consequent effects of abnormal follicular maturation and enhanced ovarian androgen production.

Present data are supportive of these hypotheses, but future studies will determine whether these views prove to be correct. However, current data provide strong support for the view that the pattern of GnRH secretion is a critical factor in the regulation of differential gonadotropin synthesis and secretion in mammalian species.

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DISCUSSION

Odell, Salt Lake City. Very nice presentation, John. I'm moved to ask whether in polycystic ovarian disease, you have attempted to use a GnRH antagonist in small doses to try to just modestly inhibit the GnRH pulsation amplitude.

Marshall: If you administer a GnRH antagonist, Bill, you can very effectively reduce LH secretion and the excess steroid production over a period of about three weeks. The problem in trying to gauge dose is that you either shut off the response or you don't. Our data have suggested a major influence on LH synthesis of GnRH pulse frequency. So even if small GnRH pulses got through at a rapid frequency, the gonadotrope cell appears to be able to recognize fast frequency signals and translate them into LH gene expression.

Wilber, Baltimore: Very lovely presentation, John. I have two questions. First, is there any role of the amplitude of the pulse, vis-a-vis the pulse frequency in gonadotropin secretory regulation? Second, is it possible in man to relate peripheral GNRH concentrations to hypothalamic events or does GNRH in the periphery derive from noncentral sources?

Marshall: I'll take the second one first, peripheral GnRH. I think most sources feel these measurements are not reflecting GnRH, which is secreted centrally. I think most of these measurements may reflect difficulties with our assays as opposed to true GnRH in plasma. The first question: yes, the amplitude does. I did not emphasize that today. If you look at gene expression, at both the transcriptional level and steady state message, the alpha and the FSH beta gene appear to be expressed as a function of pulse frequency, and are independent of pulse amplitude. The LH beta gene has a clear amplitude optimum. This is of interest in that the optimum amplitude, at least in the way we did the studies, is very similar to the amplitude of endogenous GnRH pulses that are found during the mid-cycle ovulatory surge. Indeed, the transcription of mRNA does go up markedly just before that major LH secretory surge.

Kohler, Portland: John, you made a very nice presentation. By the way, you may have mentioned this, but exogenous opioids certainly don't completely inhibit reproduction since addicts and users still get pregnant on occasion. What do they do in your system? Do they suppress the GNRH peaks?

Marshall: Yes. Some of the earliest studies of female heroin addicts showed that many of those women were anovulatory and/or amenorrheic. The problem was that there were so many other factors, like weight loss, life styles and such, that the mechanism was never quite clear. Data in primates, also in humans during some psychiatric addiction studies, have shown that the opioids do exactly what you would suspect; they shut down the frequency of GnRH secretion very efficiently.