Current Concepts of the Endocrine Characteristics of Normal Menstrual Function: The Key to Diagnosis and Management of Menstrual Disorders

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Disorders of menstrual function are among the more common problems confronting the gynecologist. The often chaotic nature of menses in the perimenarchal period soon gives rise to a cyclic, predictable pattern of menstrual bleeding, frequently accompanied by an equally consistent sequence of somatic symptoms, characteristic in the individual patient. A subsequent digression from the normal pattern will frequently prompt even the stoic to seek consultation. It is the orderly sequence of hormonal events culminating in approximate monthly ovulation that is responsible for the consistent and predictable nature of the menstrual cycle. Absent, infrequent, irregular, and otherwise abnormal menses have diverse causes but most often are an expression of a dysfunctional ovulatory mechanism. The diagnosis and management of abnormalities of menstrual function must therefore be based upon an understanding of the physiology of the normal ovulatory cycle.

The process of cyclic follicular development, selection of a dominant follicle, ovulation, and subsequent luteal function require that neuroendocrine control mechanisms be coordinated with endocrinologic and morphologic events in the ovary. Normal menstrual function further requires that the appropriate sequence of hormonal signals be integrated with events in the endometrium, which responds with prog-

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ressive growth and differentiation in preparation for potential nidation in every cycle. The character and pattern of menses, in effect, reflect the relative integrity of the various mechanisms involved in regulating ovulatory function. Understanding the manner in which hypothalamic-pituitary control of gonadotropin secretion is coordinated with ovarian steroidogenesis and follicular development allows interpretation of the ultimate endometrial response. To this end, we have reviewed the current knowledge of the endocrine characteristics of the menstrual cycle.

Hormonal Control of Follicular Development

Ovarian estrogen production has been conclusively demonstrated as the primary determinant of the cyclic pattern of gonadotropin secretion observed in the normal cycle.[1] Thus, to begin, we must first examine the mechanisms involved in the production of estrogen and its source, the ovarian follicle.

The Primordial Follicle

Each ovarian follicle, whether or not ultimately one of the select few destined to ovulate, begins as a primordial follicle, consisting of an oocyte arrested in the diplotene stage of meiotic prophase, surrounded by a single layer of granulosa cells (Fig. 1A). The initiation of follicular growth is a continuous process, independent of gonadotropin influence. It occurs at all ages, even in the prepubertal female, and remains uninterrupted during pregnancy.[2] The rate at which inactive follicles begin to grow appears to be directly proportional to the number of follicles remaining, therefore decreasing with advancing age. Although the stimulus for the initiation for follicular growth is unclear, normal cellular differentiation and progressive development depend on both the gonadotropins and ovarian steroidogenesis. Without pituitary support, the follicle can achieve only early preantral development.

The Preantral Follicle

Once growth is initiated, the follicle progresses to the preantral stage. The oocyte enlarges and is surrounded by a membrane, the zona pellucida. Cellular differentiation begins as granulosa cells undergo a multilayer proliferation and a thecal layer organizes from the surrounding stroma (Fig. 1B).

Even at this early stage of development, the enzymatic machinery necessary for steroid hormone production is already in place. Indeed, the granulosa cells of the preantral follicle have the ability to synthesize all three classes of steroids, albeit in limited quantities. However, substantially more estrogen than either androgen or progestin is produced.[3,4] Ovarian estrogen is produced through the action of the aromatase enzyme complex, which serves to convert androgens to estrogens. Aromatization is induced through the action of follicle-stimulating hormone (FSH), which first binds to specific protein receptors, present on the membranes of preantral granulosa cells.[5,6] In the presence of FSH, the preantral follicle can aromatize sufficient amounts of androgen to generate its own estrogenic microenvironment.[4] Estrogen production, in response to FSH, is limited then by the follicle's FSH receptor content. In addition to inducing aromatization, FSH acts to raise the concentration of its own receptor on granulosa cells and teams with estrogen to exert a mitogenic action, thereby stimulating granulosa proliferation.[7,8] Together, FSH and estrogen may then promote a rapid accumulation of FSH receptors, which reflects both an increase in the number of granulosa cells and a rise in the receptor density of individual cells.[7] The mechanism allows gradual expansion of the follicle's capacity for estrogen production in support of continued growth (Fig. 2).

The role of androgens in early follicular development is somewhat complex. Serving not only as substrate for FSH-induced aromatization, androgens may also bind to specific androgen receptors present in the cytoplasm of granulosa cells.[9] In so doing,

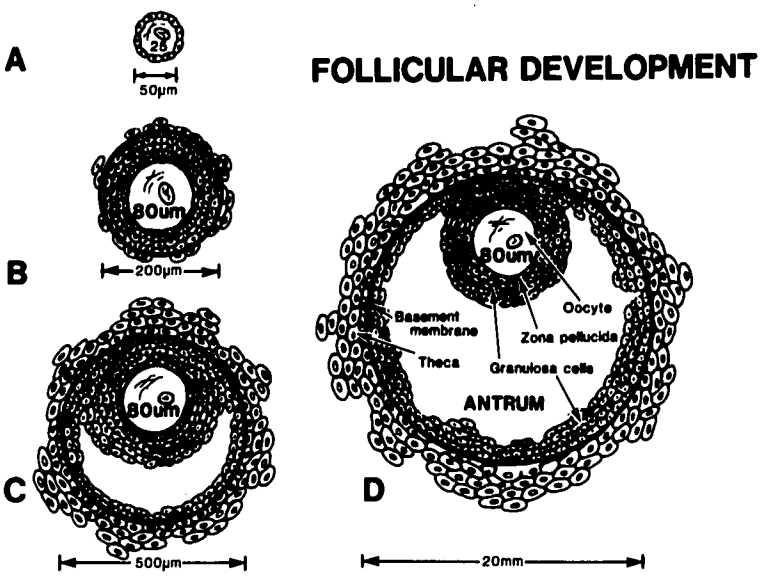

FIG. 1. *Follicular development.* **A.** *Primordial follicle.* **B.** *Preantral follicle.* **C.** *Antral follicle.* **D.** *Preovulatory follicle. (From Fritz and Speroff.*[1a] *By permission.)*

androgens may further enhance aromatase activity, an effect that can be blocked experimentally by preventing nuclear translocation of the androgen-receptor complex.[10,11] However, when placed in an *androgen-rich* environment in vitro, preantral granulosa cells favor the conversion of androstenedione to more potent androgens rather than to estrogen.[12] These products include 5α-reduced androgens such as dihydrotestosterone and androstanedione. In this form, androgens cannot be converted to estrogen and, in fact, may inhibit aromatase activity.[13] The fate of the preantral follicle is thus in rather delicate balance. At low concentrations, androgens enhance their own aromatization to estrogen. At higher levels, the still limited capacity for aromatization can be overwhelmed; the follicle becomes androgenic, and ultimately atretic. Limited development of the preantral theca, the principal source of follicular androgen, would tend to minimize the androgenic influence. Nevertheless, atresia, like the initiation of follicular

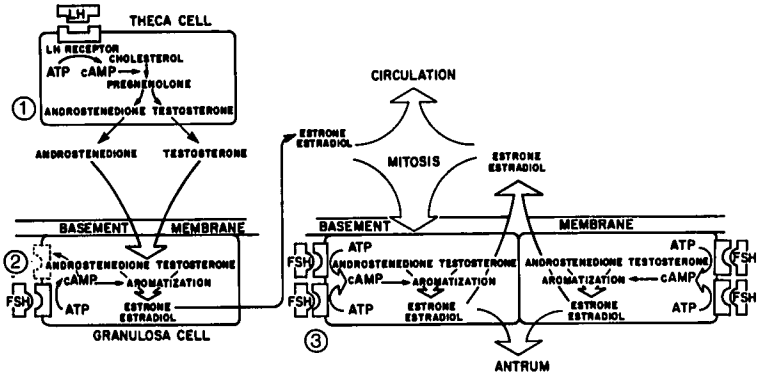

FIG. 2. *The two-cell, two-gonadotropin concept of follicular steroidogenesis. LH stimulates thecal androgen production (1). Androstenedione and testosterone are converted to estrone and estradiol through FSH-induced aromatization in the granulosa cell. FSH induces an increase in synthesis of its own receptor (2). Together, FSH and estrogen stimulate granulosa proliferation, resulting in an increase in FSH receptors and accelerating estrogen production (3). (From Fritz and Speroff.*[1a] *By permission.)*

growth, is also a continuous process. Perhaps follicles will progress in development only if emerging in an optimal gonadotropin environment. Since estrogen production in the granulosa is an FSH-mediated process and thecal androgen production is primarily luteinizing-hormone (LH)-dependent, it may be that a new cohort of follicles is successfully recruited only when FSH is elevated and LH is low. Those rising late in the luteal phase or early in a subsequent cycle may be favored by an environment in which aromatization in the granulosa can prevail.

A similar mechanism may underlie the aborted follicular development characteristic of the polycystic ovary. The androgen-rich environment of the polycystic ovary is not conducive to granulosa proliferation. As a result, FSH receptor development, aromatization, and estrogen production are limited. Rather than developing normally, follicles are prone to atresia, contributing to further enlargement of the stromal compartment, which may then be driven to produce still more androgen by often elevated LH concentrations.

Summary. Significant ovarian steroid hormone production begins early in preantral development. Follicular estrogen production occurs as a result of the FSH-induced aromatization of androgen in the granulosa cell. The capacity for estrogen production in the follicle grows as FSH and estrogen combine to promote granulosa proliferation. The creation of an estrogenic microenvironment is essential for continued follicular growth and may be possible only in those follicles emerging in the cyclic presence of optimal gonadotropin concentrations.

The Antral Follicle

Under the continuing influence of estrogen and FSH there is an increase in the production of follicular fluid that accumulates in the intercellular spaces of the granulosa, eventually coalescing to form a cavity as the follicle makes its gradual transition to the antral stage (Fig. 1C). With the formation of the antrum, the follicular fluid provides a means whereby the oocyte and surrounding granulosa cells can be nurtured in an endocrine environment unique to each follicle.

Neither FSH nor LH is usually detectable in antral fluid unless gonadotropin levels are elevated in plasma.[12] LH is normally not present in follicular fluid until or just after the midcycle surge. If levels of LH are prematurely elevated in plasma and antral fluid, intrafollicular androgen levels rise and degenerative changes appear as mitotic activity in the granulosa declines.[12] Here again, evidence suggests that the early antral follicle, like its preantral predecessor, is likely to progress only if it is developing under appropriate tropic hormone stimulation.

The presence of estrogen and FSH in antral fluid is an essential requirement for sustained accumulation of granulosa cells and continued follicular growth.[14] Whereas an estrogenic environment supports granulosa proliferation, FSH responsiveness, and aromatization, an androgenic environment antagonizes estrogen-induced granulosa proliferation and, if sustained, promotes degenerative changes in the oocyte. Antral follicles with the greatest rates of granulosa proliferation contain the highest estrogen concentrations, the lowest androgen/estrogen ratios, and are most likely to house a healthy oocyte. Recently, the number of granulosa cells in the deoxyribonucleic acid (DNA) S-phase of mitosis, an indication of the proliferative activity of the cell population, has been shown to correlate well with greater estrogen concentrations and lower androgen/estrogen ratios in follicular fluid.[15] Ongoing efforts to improve the efficiency of in vitro fertilization and embryo transfer have identified the need for such an objective measure of the relative health and viability of the oocyte. Furthermore, experience to date suggests that oocytes that subsequently give rise to successful pregnancies are most often recovered from follicular fluid aspirates having this same steroid profile.[16]

The steroids present in antral fluid are

found in concentrations often several orders of magnitude higher than those in plasma and no doubt reflect the functional capacity of the surrounding granulosa and thecal cells.[17] The synthesis of steroid hormones appears to be functionally compartmentalized within the follicle. Although each component retains the ability to produce progestins, androgens, and estrogens, granulosa cells exhibit a preferential production of estrogen while androgen synthesis predominates in the theca.[4,13,18,19] FSH receptors, which mediate the induction of aromatization, are not detectable on thecal cells.[20] As a result, aromatase activity in the granulosa far exceeds that in the theca. However, in vitro studies of both isolated and recombined granulosa and theca tissue have actually demonstrated a cooperative effort toward steroid hormone production, now known as the two-cell, two-gonadotropin concept of ovarian steroidogenesis. In response to LH, thecal tissue is stimulated to produce androgens that, upon diffusion to the granulosa, can then be converted to estrogens through FSH-induced aromatization (Fig. 2).[4,19,21-24]

The local interaction between the granulosa and the thecal compartments, which results in accelerated estrogen production, does not appear to be fully functional until later in antral development. Like preantral granulosa cells, the granulosa of small antral follicles still exhibit an in vitro tendency to convert significant amounts of androgen to the more potent 5α-reduced form. In contrast, granulosa cells isolated from large antral follicles readily and preferentially metabolize androgens to estrogen.[12] Early in development, the relative balance between reductase and aromatase activity may affect the ability of the follicle to generate an estrogenic milieu in support of continued growth. Later, the rapid and progressive accumulation of FSH receptors in the granulosa of the growing follicle facilitates the aromatization of androgen derived from an enlarging thecal compartment. Thereafter, the combined effort of both compartments results in the more efficient production of estradiol necessary to generate the preovulatory estrogen surge. Thus, relative dependence on a coincident, favorable gonadotropin environment would appear to persist until such time as the follicle acquires sufficient size and steroidogenic capacity to produce estrogen in quantities capable of influencing gonadotropin secretion itself.

Selection of the Dominant Follicle

As the antral follicle grows, the interaction between estrogen and FSH, so crucial in promoting and supporting its gradual maturation, may now also play a central role in the selection of the follicle destined to ovulate. With rare exception, only a single follicle will ovulate in each ovarian cycle. The "ovulatory quota" is maintained with striking consistency and can be reliably overridden only with the administration of exogenous gonadotropins. This suggests that modulation of gonadotropin secretion is involved in the "selection" of a dominant follicle. While estrogen exerts a positive influence on FSH action within the maturing follicle, its negative feedback relationship with FSH release at the hypothalamic-pituitary level may serve to withdraw gonadotropin support from other, less developed follicles.[25] A fall in FSH levels may lead to a decline in FSH-dependent aromatase activity, thereby limiting estrogen production in less mature follicles, with subsequent interruption of granulosa proliferation the inevitable consequence. Ultimately, such a sequence of events can only result in the conversion of the once estrogenic follicular microenvironment to one that is androgenic in nature, thereby inducing irreversible atretic changes.

An asymmetry in ovarian estrogen production, presumably an expression of the emerging dominant follicle, can be detected in ovarian venous effluent as early as the 5th to the 7th day of the cycle, corresponding with the gradual fall of FSH levels observed at the midfollicular phase (Fig. 3).[26] Such negative feedback of estrogen on FSH appears to inhibit the growth of other follicles with considerable effectiveness. The

premature elevation of circulating estrogen levels early in folliculogenesis results in FSH suppression and a prolonged follicular phase.[25] Exogenous estrogen, administered after selection of the dominant follicle, disrupts preovulatory development and induces atresia.[27] By cycle day 7, after removal of the dominant follicle, there are no follicles that still retain the ability to respond to exogenously administered gonadotropins.[28] Similarly, by cycle day 9, after ablation of the dominant follicle, no other follicles remain sufficiently developed to substitute and allow ovulation to occur on time.[29] The selective suppression of FSH during the follicular phase, and even in the immediate preovulatory interval, may result in atresia, followed by recruitment and selection of a new follicle.[30]

Paradoxically, whereas the sensitivity of FSH secretion to the negative feedback of estrogen may serve to inhibit the growth of all but the dominant follicle, the selected follicle itself remains dependent on FSH and must complete its preovulatory development in the face of declining plasma levels. The dominant follicle must somehow retain a unique responsiveness and escape the consequences of FSH suppression, induced by its own accelerating estrogen production. A rate of granulosa proliferation surpassing that of other follicles in the cohort gives the dominant follicle the advantage of relatively greater FSH receptor content. As a result, the stimulus for aromatization can be maintained while it is withdrawn from less developed follicles. Indeed, soon the selected follicle develops a capacity for estrogen production exceeding the collective contributions of the other follicles. In addition, its accumulation of a greater mass of granulosa cells is accompanied by advanced development of the thecal vasculature. By cycle day 7, the administration of fluorescent human chorionic gonadotropin (hCG) can demonstrate dense thecal uptake only in the follicle emerging as morphologically dominant.[31] By day 9, thecal vascularity in the dominant follicle is twice that of other antral follicles.[32] Such advanced vascularization may offer a preferential delivery of FSH to the follicle also possessing the greatest number of FSH receptors. These events may allow the dominant follicle to retain a unique FSH responsiveness and permit continued preovulatory development despite waning gonadotropin levels.

Certainly, the negative feedback relationship between estrogen and FSH secretion seems a likely mechanism whereby the dominance of the selected follicle can be maintained and further enhanced, once established. However, the mechanism cannot, on its own, account for the initial selection of only a single follicle. After all, the dominant follicle cannot produce sufficient estrogen to influence gonadotropin secretion until after it is selected. The normal ovulatory quota of one is maintained even when selection occurs under augmented FSH stimulation. Despite development in the presence of FSH concentrations significantly above those normally observed early in the follicular phase, the cohort of follicles recruited following luteectomy in hemiovariectomized animals yields but a single dominant follicle.[33] Under such circumstances, FSH levels nevertheless steadily decline once a dominant follicle is selected. In contrast, the multiple ovulations frequently observed following administration of exogenous gonadotropins occur in association with sustained elevations of FSH which persist even to the time the ovulatory stimulus of hCG is administered. A striking degree of bilateral ovarian hyperstimulation was also observed when pure FSH at supraphysiologic levels was administered throughout the follicular phase.[34] Thus, although perhaps not the sole mechanism of initial selection, the gradual withdrawal of gonadotropin support induced by the negative feedback of estrogen on FSH does appear to be one means whereby only the selected follicle will ovulate in each cycle.

The initial process of selection may involve a similar, selective suppression of pituitary FSH secretion, although not as a result of the negative feedback of estrogen. It

appears that estrogen is not the sole agent modulating FSH secretion during the follicular phase. Folliculostatin is a peptide moiety produced within the follicle and secreted into the follicular fluid and ovarian venous effluent. As a product of granulosa cells, its concentration in antral fluid increases with follicular size.[35] Alternatively known as "inhibin," folliculostatin is capable of selectively suppressing FSH secretion from the pituitary gland.[36] Its absence has been suggested as the reason that gonadotropin levels in the castrated or postmenopausal woman cannot be completely normalized with any level of exogenous hormone replacement. Porcine follicular fluid, a source of inhibinlike activity, lowers basal FSH concentrations when administered to castrated female monkeys and pretreatment with porcine follicular fluid inhibits FSH release in response to a bolus of exogenous gonadotropin-releasing hormone (GnRH).[37] Inhibinlike activity has also been demonstrated in human follicular fluid. Of interest are preliminary investigations that have detected its presence in antral fluid obtained only in the follicular and not the luteal phase.[38] It is hypothesized that enhanced secretion of folliculostatin from the cohort of follicles recruited in the early follicular phase may serve to limit FSH release and decrease follicular stimulation.[39] The balance between FSH and folliculostatin may limit the size of the emerging cohort, prevent hyperstimulation, and commence the process of selection. The follicle fortunate enough to have achieved perhaps even the slightest developmental edge may seize the advantage to emerge as dominant, then express and maintain its dominance through continued elaboration of folliculostatin and increasing quantities of estrogen. The modulation of pituitary FSH secretion, perhaps initiated by the folliculostatin collectively secreted by the granulosa of all the follicles in the cohort, may then continue and become further enhanced as the negative feedback of estrogen from the emerging single dominant follicle compensates for the progressive decline in folliculostatin production that results from the gradual lapse of smaller follicles into atresia. Thus, a gradual withdrawal of gonadotropin support may be the mechanism through which a single follicle both first acquires and then maintains dominance over other follicles in the cohort.

The dominant follicle so selected may then take further advantage of its greater FSH receptor content and the privileged delivery of gonadotropins provided by advanced development of its thecal vasculature. FSH induces LH receptor development on the granulosa cells of larger antral follicles (Fig. 4).[40] Acting through cyclic adenosin 3′, 5′ monophosphase (cAMP), FSH stimulates the appearance of LH receptors in a time and dose-dependent manner.[41] The rate of appearance of LH receptors also increases markedly with increasing exposure to estrogen.[42] Having the capacity for continued response to declining levels of FSH, which also allows it alone to maintain high local estrogen concentrations, the domnant follicle thus enjoys optimal conditions for LH receptor development. Its accelerating estrogen production, which later acts centrally to stimulate the LH surge, now acts locally to promote induction of the receptors required for a response.

Other Regulatory Mechanisms

In addition to inducing LH receptor development, FSH has been shown to induce specific prolactin (PRL) receptors on granulosa cells in the rat.[43] PRL clearly appears to interfere with FSH-induced aromatization in this species.[43] PRL is always present in follicular fluid, although concentrations progressively decrease during folliculogenesis and are lowest in the preovulatory follicle.[44] Anovulatory, hyperprolactinemic women may be refractory to exogenous gonadotropins, although pituitary sensitivity to GnRH appears to remain intact.[45,46] This suggests that PRL may exert an inhibitory influence at the level of the ovary. Indeed, high-affinity-PRL receptors have been demonstrated on the membranes of

human follicular elements.[47] PRL is capable of inhibiting FSH-induced aromatization and it suppresses both basal and hCG-stimulated steroidogenesis in the human ovary in vitro.[48,49] In addition, serial ultrasound in cycling women with metaclopromide-induced hyperprolactinemia has demonstrated a decrease in size of the largest follicle in association with reduced steroid production.[50] However, PRL concentrations in human follicular fluid remain at low levels even in the presence of significant hyperprolactinemia.[51] Only when circulating levels of PRL rise to those more commonly observed in association with demonstrable pituitary adenomas are intrafollicular concentrations elevated and associated with a reduction in FSH accumulation, granulosa proliferation, and lower estradiol levels in antral fluid. Furthermore, GnRH replacement in monkeys with hypothalamic lesions induces normal ovulatory menstrual cycles despite marked elevations in circulating PRL concentrations.[52] Therefore, the existence or significance of the influence PRL exerts at the ovarian level during folliculogenesis remains unclear. Available data seem to suggest that while elevated PRL levels may indeed have an impact on follicular development, their effect is not significant in modest idiopathic or drug-induced hyperprolactinemia.

Researchers have suggested that a substance like GnRH, termed "gonadocrinin," exists in the ovary of the rat, and specific receptors for GnRH have been identified in the ovary.[53,54] The concentration of these ovarian receptors increases in response to GnRH and appear higher in follicular than in luteal tissue.[54] The peptide has been reported to inhibit LH receptor development as well as steroidogenesis in granulosa cells.[54] Not surprisingly, a role for such a locally produced GnRH-like peptide has been hypothesized in the process of folliculogenesis and ovarian hormone production. However, recently the FSH-induced steroid production of granulosa cells derived from healthy human follicles was observed to progress unaffected by the addition of GnRH or an agonist in vitro.[55] The existence and source of the synthesis of an ovarian gonadocrinin is yet to be established in primates. Certainly, GnRH receptors in the ovary are unlikely targets for the minimal quantities of hypothalamic GnRH that may reach the peripheral circulation. A locally produced GnRH-like peptide may have physiologic significance and offer yet another means whereby follicular development and steroidogenesis are regulated in the ovary.

Feedback Mechanisms

The mechanisms involved in the control of follicular development obviously involve complex hormonal interactions within the ovary, but it is the feedback relationships of the ovarian steroids with pituitary gonadotropins that allow the progress of events in the ovary to be coordinated with higher centers in the hypothalamus and pituitary. Primarily through its estrogen production, the dominant follicle can assume control of its own destiny. By altering gonadotropin secretion through feedback mechanisms, it can optimize its own environment to the detriment of other follicles.

Our earlier dicussion illustrated the negative feedback effect of estrogen on the release of FSH. Even at low levels, the release of pituitary FSH is exquisitely sensitive to the inhibitory influence of estrogen and responds almost immediately. At higher levels, suppression of FSH is profound and sustained.

In contrast, the influence of estrogen on LH release varies with concentration and duration of exposure. At all levels, like its action on FSH, estrogen commands a negative feedback relationship with LH. At higher levels, however, estrogen is also capable of exerting a positive feedback effect on LH release, a response dependent on both the strength and duration of the estrogen stimulus.[1] In order to exert a positive feedback stimulus sufficient to induce the preovulatory LH surge, estradiol must rise above a critical threshold level which, in

women, approximates 200 pg/ml.[56] In addition, once threshold levels of estrogen are attained, they must be maintained for up to 50 hours or more to become effective.[56]

The strength/duration characteristics of the positive feedback mechanism have been aptly demonstrated in the rhesus monkey.[1] Estrogen concentrations below the apparent threshold are unsuccessful in inducing an LH surge even when maintained for up to 120 hours. Above the threshold, estrogen also fails if maintained for less than 36 hours. Levels above the threshold but in the lower physiologic range result in a delay in the onset of the LH surge, whereas supraphysiologic levels advance surge onset. The estrogen stimulus must be applied until after the surge actually begins. Otherwise, the LH surge is abbreviated or fails to occur at all.[1]

It is interesting to note that when human menopausal gonadotropins (hMG) are administered to women with normal endocrine characteristics for the purpose of inducing multiple preovulatory follicles for subsequent oocyte retrieval and in vitro fertilization, hCG is usually required to achieve the final stages of maturation.[39] Seldom are spontaneous LH surges observed despite the persistence of normal threshold levels of estradiol for several days. Observations of the response of normal women to exogenous gonadotropins support the theory of the existence of folliculostatin in man and suggest that it may play a role in midcycle feedback dynamics. The suppressive action of folliculostatin on the pituitary gonadotrope may not be limited to inhibition of FSH alone. Pretreatment with porcine follicular fluid negates the gonadotropin surges normally observed in intact monkeys given an estrogen challenge during the follicular phase.[39] When hMG is administered to ovariectomized animals and is followed by an estrogen challenge, the normal estrogen positive feedback response is observed.[39] Together, the results suggest that the blockade of the positive feedback response that may occur during hMG therapy requires intact ovaries. Perhaps the decline in folliculostatin production that may accompany the atresia of all but the dominant follicle is a necessary prerequisite for threshold levels of estradiol to induce the characteristic surge. Exogenous gonadotropins, by increasing the size of the cohort of follicles recruited and by supporting development of more than a single dominant follicle, might well induce folliculostatin production in excess of the production normally observed. The presence of supraphysiologic levels of folliculostatin, which would also be likely to persist during preovulatory development, may interfere with the normal positive feedback response. Verification of such a mechanism must await a more precise measurement and actual characterization of folliculostatin.

Summary. The cohort of follicles recruited to participate in each new ovarian cycle is likely to consist of those follicles whose growth was initiated coincident with the cyclic appearance of an optimal high FSH, low LH, gonadotropin environment. Folliculostatin produced in the granulosa may limit the size of the cohort from which a single follicle is normally selected. As the first in the cohort to achieve sufficient size for efficient aromatization of androgens, the selected follicle may then express its dominance through production of estrogen in quantities capable of influencing its own gonadotropin environment. The negative feedback of estrogen produced by the dominant follicle effectively suppresses pituitary FSH secretion and serves to withdraw gonadotropin support from the other follicles in the cohort. The same developmental advantage which permits the follicle to emerge as dominant allows it to retain a unique responsiveness to the decline in FSH levels induced by its own estrogen production. FSH is then able to induce the appearance of LH receptors, an effect enhanced by the same accelerating estrogen production that will ultimately achieve and maintain the threshold concentrations necessary to effect a positive-feedback response and generate the LH surge.

The Preovulatory Follicle

As the follicle undergoes its final maturation, granulosa cells enlarge and acquire lipid inclusions. The theca becomes vacuolated and richly vascular, giving the preovulatory follicle a hyperemic appearance. The oocyte within resumes meiosis and approaches completion of its reduction division (Fig. 1D).

Now clearly singular and dominant, the preovulatory follicle continues to produce ever-increasing amounts of estrogen. As a result, estradiol levels rise rapidly, surging to a peak approximately 24–36 hours prior to ovulation.[57] Consequently, FSH declines gradually to its nadir just prior to the combined midcycle gonadotropin surge (Fig. 3). Sustained threshold concentrations of estradiol stimulate the LH surge, thus inducing a quantitative elevation of gonadotropins just as LH-receptor development and ovarian responsiveness reach their peak. In the absence of either FSH or adequate estrogen, follicles respond to an LH bolus with atresia rather than luteinization and, in the monkey, premature administration of hCG disrupts preovulatory development and results in the failure of ovulation.[42,58] In providing the ovulatory stimulus to the selected follicle, the LH surge may then also serve to seal the fate of any remaining lesser follicles.

Acting through its receptor, LH promotes luteinization of the granulosa. Within the preovulatory follicle, estradiol and LH interact in a synergistic fashion in order to stimulate cholesterol side-chain cleavage activity.[59] The result is an accelerated production of pregnenolone that, as the immediate steroidogenic precursor, promotes a rise in progesterone synthesis. An increase in progesterone production can be detected in the venous effluent of the ovary bearing the preovulatory follicle 24–48 hours before ovulation.[26] A significant rise in circulating levels of progesterone occurs on the day of the LH peak, 12–24 hours prior to ovulation.[60] This small but significant increase in the production of progesterone in the preovulatory period has immense physiologic importance.

The injection of progesterone in the presence of otherwise subthreshold levels of estradiol can induce a characteristic LH surge.[61] When progesterone is administered after levels of estradiol sufficient to induce an LH surge have already been imposed, the resulting LH surges occur earlier, reach greater amplitude, and are shorter in duration than those observed in the absence of progesterone.[62] In women, there is compel-

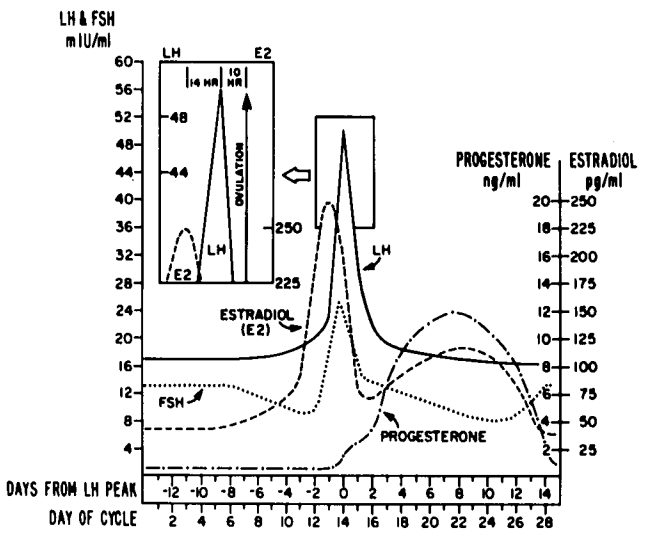

FIG. 3. *The temporal relationship of gonadotropin and ovarian steroid secretion in the normal menstrual cycle. (From Fritz and Speroff.*[1a] *By permission.)*

ling evidence that without the preovulatory rise in progesterone, the midcycle FSH peak, which normally accompanies the LH surge, does not occur at all.[63,64]

Progesterone affects the positive feedback response to estrogen in both a time- and dose-dependent manner. Progesterone will advance and enhance the positive feedback response only if introduced after adequate estrogen priming. When administered either before an adequate estrogen stimulus or in high doses coincident with threshold levels of estradiol, progesterone blocks the midcycle LH surge.[63,65,66] Thus, the preovulatory rise in progesterone production serves to both augment the positive feedback of estradiol and induce a combined midcycle LH/FSH surge. Acting through the sequential feedback signals of both estradiol and progesterone, the preovulatory follicle may then communicate its full maturity to higher centers and coordinate its final development with the ovulatory stimulus.[61]

Summary. The final developmental progress of the preovulatory follicle is marked by a level of estrogen production sufficient to achieve and maintain peripheral threshold concentrations of estradiol that induce the LH surge. Rapidly rising levels of LH act through the LH receptors previously induced by FSH and estrogen to initiate luteinization of the granulosa. The resulting preovulatory rise in progesterone production then facilitates the positive-feedback action of estrogen already in progress and serves to synchronize final follicular maturation with the actual ovulatory stimulus.

Ovulation

It now seems clear that the preovulatory follicle, through its steroid hormone production, can initiate and control the ovulatory stimulus. The sequence of events that follows is not yet well defined but appears to be initiated by the massive release of LH triggered by sustained threshold levels of estradiol.

Recent efforts in the area of in vitro fertilization have made it necessary to accurately predict the time of ovulation. Considerable variation exists from cycle to cycle, even in the same patient. Observations made by several investigators place ovulation approximately 10-12 hours after the LH peak and 24-36 hours after peak estradiol levels are attained (Fig. 3).[57,67,68] The onset of the LH surge appears to be the most reliable indicator of impending ovulation, occurring 28-32 hours before follicle rupture.[67,68] In addition to stimulating the luteinization of granulosa cells, it appears that the LH surge prompts the resumption of meiosis in the oocyte and promotes the synthesis of prostaglandins essential to follicle rupture.[69-71]

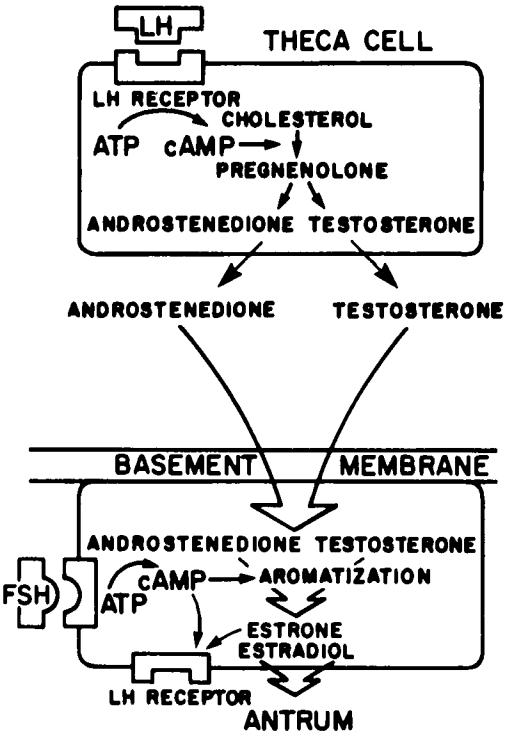

FIG. 4. *LH receptor development in the granulosa. Enhanced by estradiol, FSH induces LH receptors on the granulosa cells of larger antral follicles.*

As LH levels rise, tissue concentrations of cAMP increase in the preovulatory follicle.[72] The LH-induced increase in cAMP activity appears to mediate both oocyte maturation and luteinization of the granulosa (Fig. 5).

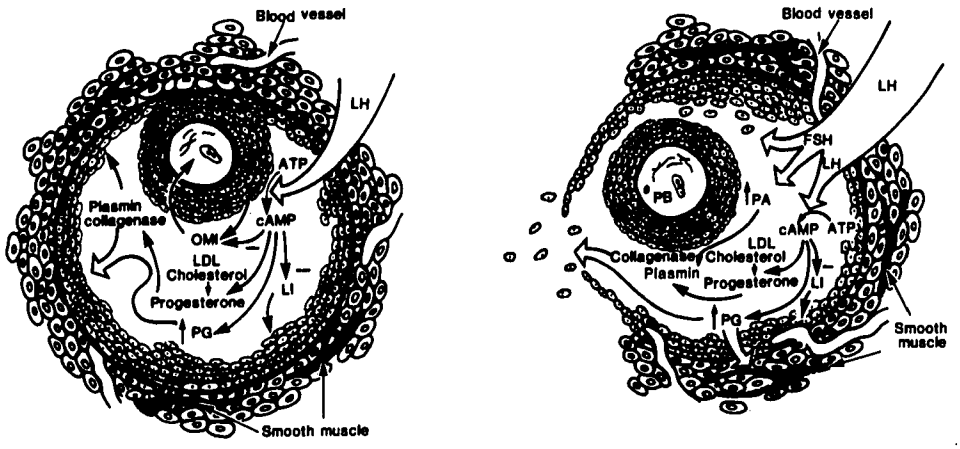

FIG. 5. *The Mechanism of ovulation. Rising LH Levels stimulate an increase in cAMP. cAMP mediates luteinization and resumption of meiosis, overcoming the action of local inhibitors, luteinization inhibitor (LI) and oocyte maturation inhibitor (OMI). As luteinization proceeds, progesterone levels rise, enhancing the activity of proteolytic enzymes and increasing follicle wall distensability. Prostaglandin (PG) levels increase and, together with plasmin and collagenase, may serve to digest the follicle wall. The midcycle LH surge brings about completion of reduction division and formation of the first polar body (PB). Midcycle FSH stimulates expansion of the cumulus and production of plasminogen activator (PA). Continued enzymatic digestion results in follicle wall rupture. Prostaglandins (PG) may stimulate contraction of smooth muscle in the theca externa, causing oocyte expulsion. Branching vessels penetrate the luteinized granulosa. (From Fritz and Speroff.*[1a] *By permission.)*

Resumption of meiosis can be induced in follicles given direct injections of dibutyryl cAMP.[69] Rising levels of the cyclic nucleotide also parallel an increase in progesterone production in the luteinizing granulosa.[70] cAMP does not appear to act directly but seems to overcome the local inhibition of both meiosis and luteinization (Fig. 5).

Oocyte maturation inhibitor (OMI) and luteinization inhibitor (LI) are two nonsteroidal inhibitors present in follicular fluid that may serve to prevent premature oocyte maturation and luteinization.[36] They differ from folliculostatin because they act locally rather than through a central inhibitory mechanism. OMI is a low-molecular-weight peptide that appears to inhibit oocyte maturation through an action on cumulus cells.[73] Its synthesis may be regulated by the steroid/gonadotropin environment. Preliminary studies of human follicular fluid aspirates have suggested an inverse correlation between the OMI concentration and the estradiol content and size of the follicle.[73] LI appears to exist in the follicular fluid of immature follicles where it may interfere with FSH induction of LH receptors. The follicular fluid of small follicles can inhibit LH receptor development and subsequent LH-induced progesterone production in vitro.[73] In contrast, follicular fluid extracted from larger follicles enhances progesterone synthesis. A better appreciation of the roles of OMI and LI must await the characterization and accurate measurement of these local nonsteroidal inhibitors.

With the LH surge, levels of progesterone in the preovulatory follicle continue to rise up to the time of ovulation.[74] The progressive rise in progesterone during the periovulatory period may act to terminate the LH surge as negative feedback effects are exerted at higher concentration.[62] In addition, progesterone may serve to increase the distensibility of the follicle wall.[74] A change in the elastic properties of the follicle wall

seems necessary to explain the rapid increase in follicular fluid volume that occurs just prior to ovulation, unaccompanied by any significant change in intrafollicular pressure.[75] The preovulatory follicle reaches a diameter of 18-25 mm as it burgeons forth from the surface of the ovary. Researchers have suggested that LH, acting through stimulation of cAMP activity or progesterone production, or both, may enhance the activity of proteolytic enzymes, thereby causing the digestion of collagen in the follicular wall and increasing its distensibility (Fig. 5).[76,77] Proteolytic enzymes such as collagenase and plasmin are present in follicular fluid and are capable of increasing follicle wall distensibility in vitro.

The LH surge also appears responsible for stimulating the local synthesis of prostaglandins. Concentrations of both prostaglandin E (PGE) and prostaglandin F (PGF) increase markedly in the preovulatory follicle or after hCG administration and are highest at ovulation, thereby suggesting a role in the ovulatory process.[71] Indeed, inhibition of prostaglandin synthesis may block follicle rupture without affecting the other LH-induced processes of luteinization and oocyte maturation.[78-80] Treatment with indomethacin can prevent ovulation and result in a luteinized, unruptured follicle. The mechanism through which prostaglandins may induce follicle rupture is unknown. They may act to free lysosomal enzymes to digest the follicular wall. However, smooth muscle cells have been identified in the ovary, and PGF stimulates ovarian "contractions."[81,82] Located in the theca externa, smooth muscle fibers may play a role in extrusion of the oocyte-cumulus cell mass (Fig. 5).[83]

As LH reaches its peak, circulating levels of estradiol plunge (Fig. 3). In a number of endocrine systems, prolonged exposure to a high concentration of hormone results in a decrease in the response of the target tissue. Such a "down-regulation" phenomenon, acting on LH receptors, may explain the precipitous fall in estradiol levels at midcycle. In fact, thecal tissue derived from healthy antral follicles exhibits marked suppression of steroidogenesis when exposed to high levels of LH, whereas exposure over a low range of concentrations stimulates steroid production.[18]

The midcycle LH surge is accompanied by a simultaneous release of FSH, although of a lesser magnitude (Fig. 3). The FSH peak is dependent on the preovulatory rise of progesterone and is probably a response to a common releasing factor, GnRH. Midcycle FSH release, however, is far more than coincidental. Mounting evidence suggests several possible functions. Plasmin is an active proteolytic enzyme involved in the breakdown of the follicle wall and is produced by the conversion of its inactive precursor, plasminogen. The synthesis of plasminogen activator, the enzymatic catalyst of conversion, is more sensitive to FSH than LH stimulation.[84] In the mouse, FSH, but not LH, stimulates mucification of the cumulus cells supporting the oocyte within the follicle (Fig. 5).[85] Cumulus expansion allows the oocyte-cumulus cell mass to become free-floating just before follicle rupture. The process involves the deposition of a hyaluronic acid matrix, the synthesis of which is stimulated by FSH in vitro.[86] Perhaps most importantly, the induction of LH receptors on granulosa cells is a specific FSH-mediated action and a necessary prerequisite for the normal progress of luteinization and subsequent synthesis of progesterone. A high incidence of a shortened or inadequate luteal phase is observed in cycles when FSH levels are low or selectively suppressed.[30]

Summary. The LH surge stimulates completion of reduction division in the oocyte, luteinization of the granulosa, and synthesis of progesterone and prostaglandins. Progesterone enhances the activity of proteolytic enzymes responsible, together with prostaglandins, for digestion and rupture of the follicle wall. The progesterone-dependent, midcycle rise in FSH serves to free the oocyte from follicular attachments and induces sufficient LH receptors to ensure adequate progesterone production in the subsequent luteal phase.

The Luteal Phase

After ovulation, the wall of the follicle becomes convoluted as the antrum fills with blood and lymph. Luteal cells are derived mainly from granulosa cells that enlarge, accumulating lipid and lutein pigment. In addition, theca-lutein cells may differentiate from the surrounding theca and stroma to become part of the corpus luteum.[87] A fine network of capillaries, branching from thecal vessels, develops and penetrates the granulosa as a marked vascularization takes place.

It is clear that the corpus luteum is the principal source of luteal-phase progesterone and that granulosa cells of the preovulatory follicle are the primary functional component of luteal tissue. The greater the number of granulosa cells removed in the course of follicular aspiration for in vitro fertilization, the smaller is the secretory potential of the subsequent corpus luteum.[39] When examined microscopically, the size and number of lipid droplets in luteal cells accurately reflect the level of progesterone production throughout the luteal phase.[88] Moreover, midluteal-phase luteectomy induces a prompt fall in progesterone concentrations, both peripherally and in the ipsilateral ovarian vein, and is followed by the premature onset of menses.[29,89]

The Requirements for Normal Luteal Function

Progesterone production, which is a measure of the functional capacity of the corpus luteum, is dependent on several factors. First of all, compelling evidence is accumulating to indicate that normal luteal function requires optimal preovulatory development. The "inadequate corpus luteum" may simply reflect similarly inadequate folliculogenesis. The selective suppression of FSH during the follicular phase is associated with lower preovulatory estradiol levels, depressed midluteal progesterone production, and a decrease in luteal cell mass. Furthermore, the luteal cells obtained in such cycles exhibit a suppressed basal as well as hCG-stimulated progesterone synthesis in vitro.[90] These findings imply that the accumulation of LH receptors on granulosa cells during the follicular phase may predetermine the extent of luteinization and the subsequent functional capacity of the corpus luteum (Fig. 6).

The life span and the steroidogenic capacity of the corpus luteum also appear dependent on continued tonic LH secretion (Fig. 6). An LH antiserum, administered to monkeys in the early luteal phase, prompts a premature decline in plasma progesterone and the early onset of menses.[91] Ovulation, induced in hypophysectomized women, is followed by subnormal progesterone production and a short luteal phase. Normal luteal function can be restored only with

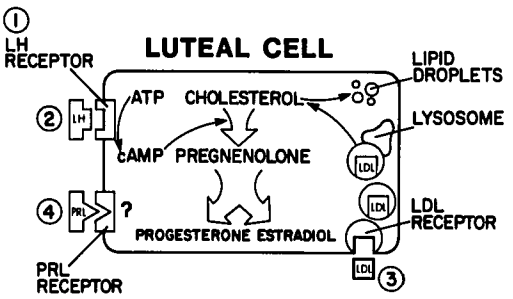

FIG. 6. *The requirements for normal luteal function. Normal luteal function and progesterone production will follow only optimal preovulatory follicular development. LH receptor development, induced by FSH and estrogen, predetermines the extent of luteinization. (1). Continued tonic LH stimulation drives luteal steroidogenesis (2). LDL-cholesterol is the obligatory substrate for progesterone synthesis in the corpus luteum and is made available as vessels penetrate the luteinized granulosa following ovulation. LDL-cholesterol enters the cell through endocytosis of the LDL/receptor complex. The LDL-containing vesicle fuses with lysosomes, releasing cholesterol for use in steroid synthesis or storage in cytoplasmic lipid droplets (3). Prolactin, in physiologic concentrations, may play a permissive role in luteal steroidogenesis (4).*

repeated doses of LH.[92] Recently, however, it was demonstrated that the length of the luteal phase in rhesus monkeys was unaffected by hypophysectomy one day following ovulation.[93] Ovulation induced with hMG/hCG or hLH 6 months after operation was similarly followed by a luteal phase of normal duration. Nevertheless, PRL levels remained detectable in these studies, thus suggesting the possibility of incomplete hypophysectomy.

Progesterone production is further dependent on low-density lipoprotein (LDL) as a source of cholesterol (Fig. 6).[94] At peak function, the mature corpus luteum produces up to 40 mg of progesterone per day during the midluteal phase.[95] The rate of de novo cholesterol synthesis is inadequate to meet such demands. As a result, the uptake and degradation of LDL is required in order for the corpus luteum to realize its full steroidogenic potential. The relatively high molecular weight of LDL limits the quantity that will diffuse into antral fluid. This relative unavailability of LDL-cholesterol in the avascular granulosa has been suggested as the factor limiting progesterone synthesis in the preovulatory follicle.[96] Following ovulation, the vascularization of the corpus luteum allows LDL-cholesterol to reach the luteinized granulosa and be used in progesterone biosynthesis. Capillary invasion of the granulosa begins within 48 hours after ovulation and reaches the central cavity by the 4th postovulatory day. Maximal capillary development and dilatation are observed at the midluteal phase, which corresponds with the peak in progesterone production.

Once available, LDL enters the cell after binding to specific membrane receptors which then undergo endocytosis. The LDL-containing vesicle is delivered to the golgi where it fuses with lysosomes. This fusion results in hydrolysis and the release of amino acids and cholesterol esters. Further hydrolysis to free fatty acids and unesterified cholesterol is accomplished by lysosomal lipase. Once released to the cytosol, free cholesterol serves not only as substrate for progesterone production but also serves as regulator for the activity of enzymes involved in de novo cholesterol synthesis and its reesterification for storage in cytoplasmic lipid droplets (Fig. 6).[95] In addition, free cholesterol is capable of controlling its own subsequent availability through down-regulation of LDL receptors.[95] Changes in progesterone production throughout the cycle are positively correlated with changes in the number of binding sites for LDL in the corpus luteum.[97] The binding capacity for LDL is greatest in luteal tissue obtained during midluteal phase. Since the number of hCG/LH binding sites and progesterone production also peak at this time, it has been suggested that LH/hCG might act to induce LDL receptors similar to adrenocorticotropic hormone (ACTH) induction of LDL receptors in the adrenal.[95,98] Further evidence that luteal progesterone production is LDL substrate-limited comes from the observation of a 10–25% cyclical fall in circulating LDL concentrations during the luteal phase.[99] Also consistent with an obligatory role for LDL in progesterone synthesis is the recent description of luteal inadequacy in a patient with abetalipoproteinemia, marked, of course, by the virtual absence of LDL cholesterol.[100]

Luteal Suppression of New Follicular Growth

Progesterone levels normally rise sharply after ovulation. They reach a peak approximately 8 days after the LH surge (Fig. 3). The presence of luteal levels of progesterone effectively inhibits new follicular growth. Progesterone replacement at luteal levels following luteectomy in intact monkeys consistently delays the next ovulation for a period matching the duration of progesterone administration.[101] Progesterone appears to exert its inhibitory influence, at least in part, at the level of the ovary.

If progesterone concentrations are monitored in ovarian venous effluents after luteectomy, ovulation in the subsequent cycle uniformly occurs on the side opposite the higher progesterone level and contralateral

to the previous corpus luteum.[102] If circulating progesterone levels are maintained following luteectomy by stimulating extraluteal progesterone production with exogenous hCG, the subsequent ovulation again occurs in the ovary with a lower progesterone concentration in its venous effluent.[103] The ovary bearing a lower, local progesterone concentration will ovulate even if it was also the site of the antecedent corpus luteum. This suggests that intraovarian progesterone may regulate new follicular growth. Indeed, progesterone may inhibit aromatization and retard estrogen-dependent folliculogenesis.[104]

In addition to acting directly at the level of the ovary, progesterone may indirectly further inhibit ovarian folliculogenesis through negative feedback on gonadotropin secretion at higher centers. A luteal suppression of gonadotropin release has been thought necessary to ensure inhibition of renewed follicular activity since exogenous gonadotropins can overcome the intraovarian inhibition of progesterone and stimulate ovulation when administered during the luteal phase.[105,106] It is important to note, however, that the elevated levels of FSH induced by luteectomy in the hemiovariectomized monkey persist despite replacment with exogenous progesterone at normal luteal levels.[33] Even under these experimental conditions, the ovary remains quiescent. New follicular growth is effectively suppressed and ovulation is delayed for a period equal to the duration of progesterone replacement. Progesterone can apparently effectively inhibit folliculogenesis even without suppressing gonadotropin secretion.

Thus, it would seem that progesterone does, in fact, act directly on the ovary to suppress new follicular growth during the luteal phase. One cannot, however, exclude a complementary action on gonadotropin secretion. Progesterone may act more to alter the pattern of gonadotropin release than to suppress it entirely. Alteration of the ratio of FSH and LH released has been suggested as a mechanism through which progesterone may act on higher centers.[107] As discussed earlier, the growth of follicles emerging in an unfavorable gonadotropin environment is most often ill-fated and largely ineffectual. Any such action would certainly be negated by hMG administration and would perhaps explain why ovulation can be induced with exogenous gonadotropins during the luteal phase. On the other hand, gonadotropins normally do fall during the luteal phase. Estrogen production, normally increasing again with progesterone after ovulation but remaining low in the progesterone-replaced, luteectomized animal, may be the reason FSH levels rise after removal of the corpus luteum despite exogenous progesterone administration. The actions of progesterone are generally dependent on the prior action of estrogen. Estrogen "priming" serves to induce development of cytoplasmic progesterone receptors. Such priming has been demonstrated in the hypothalamus and the pituitary of primates as well as in the uterus and other target tissues in the reproductive tract.[108] Perhaps the negative feedback of progesterone on gonadotropin secretion, or enhancement of that exerted by estradiol, can be expressed only after sufficient estrogen priming. In any case, the luteal decline in gonadotropin secretion would certainly further enhance any progesterone-induced inhibition of new folliculogenesis that may occur within the ovary.

Wherever the site(s) of progesterone inhibition of follicular growth may be, its action does not appear to be mediated by conversion to 17α-hydroxyprogesterone (17-OHP) or its metabolites. Whereas progesterone replacement after luteectomy will maintain normally observed levels of 17-OHP and delay ovulation, direct replacement with 17-OHP fails to inhibit new follicular growth sufficiently to significantly postpone the next ovulation.[33]

Luteolysis

In the latter half of the luteal phase, progesterone levels decline gradually, again returning to basal concentrations with the onset of menses. The luteal phase cannot be

extended indefinitely, even with progressively increasing LH exposure.[92] Apparently the corpus luteum becomes progressively less sensitive to LH stimulation. In addition, the steroidogenic capacity of luteal cells in vitro decreases with the advancing "age" of the corpus luteum.[109] One possible explanation for this involves the inhibition of LH binding. Luteal tissue contains a nonsteroidal LH-receptor binding inhibitor (LHRBI) which appears to increase in concentration throughout the luteal phase. LHRBI can inhibit progesterone secretion in vitro, and it has been implicated in the process of luteolysis.[36] An actual decrease in LH receptors would also explain the loss of LH sensitivity and reduced steroidogenic capacity observed in the latter half of the luteal phase. Indeed, the number of LH receptors in the corpus luteum gradually decreases from peak levels at midluteal phase to minimal levels at the time of menses. However, the decline in LH binding capacity begins after circulating progesterone levels have already begun to fall.[98] Since the initial stages of luteolysis are not necessarily preceded by or associated with a loss of LH receptors, a reduction in LH binding capacity would not appear to be an obligatory step in the normal induction of luteolysis.

The decline in progesterone production occurs as estradiol again rises to plateau at the midluteal phase, suggesting that estrogen may initiate luteolysis.[110] There is considerable evidence to support a role for estrogen in the decline of the corpus luteum. The premature elevation of circulating estradiol levels early in the luteal phase results in a prompt fall in plasma progesterone concentrations.[111] Direct injections of estradiol into the ovary bearing the corpus luteum induce luteolysis, whereas similar treatment of the contralateral ovary produces no effect.[112] The concentration of estrogens in the corpus luteum of both monkeys and man increases as the luteal phase progresses.[110] In vitro, estradiol inhibits the hCG-induced progesterone production of luteal cells in a dose-dependent manner.[113,114]

The mechanism of the estrogen-induced luteolysis has received considerable attention. A good deal of evidence has accumulated to implicate the mediation of prostaglandins. Estrogen-induced luteolysis can be blocked by inhibiting prostaglandin synthesis.[115,116] The corpus luteum is capable of synthesizing prostaglandins, and its ability to bind both PGE and PGF to luteal cells has been demonstrated.[117-120] PGE_2 stimulates progesterone production, whereas $PGF_{2\alpha}$ inhibits progesterone synthesis.[121-124] Both appear to operate through modulation of LH-dependent cAMP accumulation.[122,125,126] PGE can prevent the luteolytic effect of PGF both in vivo and in vitro.[127] Interestingly, there is a significant increase in the ratio of PGF to PGE in the corpus luteum during the late luteal phase.[118] Estrogen induces similar effects on relative prostaglandin concentrations when administered early in the luteal phase, thus prompting speculation that estrogen acts to tip the balance in favor of the luteolytic action of PGF.[128]

There is also evidence to suggest that estrogen-induced luteolysis is the result of its interference with the tropic action of LH. Diethylstilbestrol (DES) reduces LH binding sites in luteal tissue in vitro.[129] Estradiol, injected directly into the corpus luteum, decreases LH-receptor binding capacity without affecting binding affinity.[121] Although modulation of LH receptors in the corpus luteum could be involved in estrogen-induced luteolysis, it does not appear to be an obligatory step in spontaneous luteolysis. In addition, the mechanism of any such intraovarian inhibition of LH binding remains unclear since measurable quantities of estrogen receptor cannot be demonstrated in primate luteal tissue.[130] This fact argues convincingly that estrogen-induced luteolysis is mediated through another mechanism because the luteolytic action of exogenous estradiol can be blocked by concurrent administration of clomiphene, a known estrogen receptor antagonist.[130] Furthermore, subcutaneous estradiol implants, which achieve normal luteal levels in peripheral serum, can induce luteolysis without pro-

ducing an increase in the concentration of estradiol within the corpus luteum.[131]

The luteolytic action of exogenous estrogen may well result from a central negative feedback inhibition of LH release. The successful induction of luteolysis following either intraluteal or systemic estrogen administration is associated with a significant fall in circulating LH concentrations.[130,131] Moreover, clomiphene blockade of estradiol-induced premature luteolysis is ccompanied by the absence of a decline in LH levels.[130] Although there is no doubt that estrogen can indeed induce functional luteolysis, the question of whether estrogen is at all involved in the normally occurring demise of the corpus luteum remains. An estrogen-mediated negative feedback mechanism is certainly a plausible and attractive hypothesis. After all, the importance of a decline in LH in spontaneous luteolysis is suggested by the steady fall in circulating LH as the luteal phase progresses and as estradiol rises to its midluteal phase plateau. However, recent evidence would argue against a causal role for estrogen in the normal menstrual cycle. The administration of an estrogen antagonist during the luteal phase fails to prolong the lifespan of the corpus luteum.[130] Progesterone levels gradually decline and menstruation ensues despite the maintenance of LH levels and estrogen-receptor blockade. In addition, effective suppression of luteal phase estrogen production with an aromatase inhibitor fails to delay the onset of menses.[132] Thus, the mechanisms of spontaneous and estrogen-induced luteolysis may differ, and the cause of luteal regression in the normal cycle remains unknown.

If indeed the secondary rise in estradiol production during the luteal phase is not the stimulus for luteolysis, what function may it serve? It is unlikely that an endocrine event so distinct and consistent would reflect only the spectrum of steroid production in the mature corpus luteum. Recent evidence suggests another possibility. When the levels of both estradiol and progesterone were determined at midluteal phase in a large group of infertile patients and correlated with the results of a histologic investigation of the endometrium late in the same cycle, the findings were rather surprising.[133] As expected, the endometrium of those patients with low midluteal progesterone concentrations reflected inadequate secretory development, whether associated with normal or low levels of estradiol. Of interest was the observation of similar histologic delay in patients found to have normal midluteal progesterone levels but a depressed estradiol concentration. It is now well established that the induction of endometrial progesterone receptors is an estrogen receptor-mediated phenomenon.[134] Secretory endometrial development, a result of the action of progesterone, might then depend on estrogen induction of sufficient progesterone receptors to mediate response. The half-life of endometrial cytoplasmic progesterone receptors is relatively brief and concentrations fall abruptly with the onset of significant progesterone production following ovulation.[134,135] Continued estradiol secretion is necessary for the maintenance of progesterone receptor concentrations.[134] The secondary rise in estradiol production during the luteal phase may serve to replenish endometrial progesterone receptor levels. Without such action, perhaps secretory development may not reach maturity.

It is interesting to note that clomiphene administration has been associated with luteal insufficiency and delayed maturation of the endometrium. Since it is prescribed for anovulatory women, the lack of full endometrial development may simply reflect less than optimal preovulatory development and suggest the need for still greater stimulation of folliculogenesis. A subsequent increase in the dose of clomiphene administered may indeed accomplish further enhancement of follicular growth by inducing greater gonadotropin release. However, the "antiestrogenic" action of clomiphene is a result of its competition with estradiol for the cytoplasmic estrogen receptor and subsequent interference with estrogen-receptor replenishment.[130] Thus, higher circulating levels of clomiphene may also enhance in-

hibition of estradiol induction of progesterone receptors in the endometrium. In support of the hypothesis are the results of tamoxifen administration in a group of infertile women suspected of having luteal phase defects.[136] When administered in a manner identical to that normally recommended for clomiphene, from the 5th through 9th day of the cycle, tamoxifen significantly increased progesterone production and the length of the luteal phase when compared with untreated control cycles. Nevertheless, the incidence of delayed endometrial maturation actually increased in treatment cycles. It is tempting to speculate that administration of this potent, noncompetitive, estrogen receptor antagonist prevented normal secretory endometrial development by inhibiting estradiol induction of cytoplasmic progesterone receptors, thereby negating the influence of normally adequate progesterone concentrations. When an attempt was made to characterize the endocrine profiles of women with a short luteal phase, two distinct abnormalities were noted.[137] Women who exhibited a short luteal phase were found to have subnormal FSH concentrations during preovulatory folliculogenesis. The significance of this observation has since been demonstrated. Inadequate luteal function is consistently observed when follicular phase FSH levels are experimentally suppressed. The other endocrine aberration observed was the absence of a secondary rise in estradiol production during the luteal phase.

Certainly there is sufficient evidence to suggest a role for luteal phase estrogen production in endometrial progesterone receptor replenishment and its requirement for normal secretory development. Confirmation must await the results of investigations currently underway. Although depressed luteal estradiol levels, whether naturally occurring or experimentally induced, do not preclude maintenance of a luteal phase of normal duration, normal endometrial maturation, under such circumstances, may not occur. It is also important to note that experimental observations of "functional" luteolysis, as determined by a reduction in the level of progesterone production, may not necessarily include significant impact on the target tissue, the endometrium, or preclude its normal secretory function.

Luteal "Rescue" in the Fertile Cycle

Unless pregnancy intervenes, the demise of the corpus luteum is inevitable. hCG acts to maintain luteal function, rescuing the corpus luteum and prolonging its progesterone production until placental steroidogenesis is well established.[138-141] When administered in a manner mimicking the pattern of secretion observed following implantation, hCG significantly augments progesterone production over that observed in untreated controls.[141] Like LH, hCG appears to operate through induction of cAMP activity. hCG stimulates cAMP production in the corpus luteum, an effect most pronounced at midluteal phase.[142] In the fertile cycle, hCG first appears at the peak of corpus luteum development and, presumably, thereby prevents the onset of luteal regression.[143] The mechanism of corpus luteum rescue may involve the interference of hCG with the synthesis of prostaglandins and with the balance between their respective tropic and lytic actions. $PGF_{2\alpha}$ can inhibit hCG stimulation of cAMP and the progesterone production of the midluteal corpus luteum in vitro.[142] However, hCG inhibits prostaglandin synthesis and can prevent the characteristic increase in the PGF/PGE ratio normally observed in corpora lutea during the late luteal phase.[144]

By maintaining progesterone synthesis and elevated circulating progesterone levels, hCG is able to suppress new follicular activity, acting through the same mechanisms operative in the luteal phase of the nonfertile cycle. It appears that hCG may also exert a suppressive influence on new folliculogenesis through a mechanism not involving an action on the corpus luteum.[145] At levels typical of early pregnancy, hCG can delay the onset of the next LH surge if administered before and even after removal of the

corpus luteum, an effect not associated with any significant change in the gonadotropin environment. Although obviously exerted at an extraluteal site, the mechanism of such inhibition of follicular growth may nevertheless still involve progesterone mediation. Intraovarian progesterone levels can be maintained following luteectomy by stimulating extraluteal progesterone production with similar amounts of hCG.[103] Thus, progesterone may still be the agent of suppression under these experimental conditions. Prolongation of the follicular phase with hCG treatment after luteectomy could also be a result of hCG itself, perhaps enhanced by progesterone as well. Although no change was observed in gonadotropin levels with hCG administration, hCG is similar to LH in action and the two share the same receptor. The presence of hCG could therefore simulate a gondotropin environment in which folliculogenesis is clearly ineffectual.

The effective inhibition of new follicular growth during pregnancy may thus involve the suppressive actions of enhanced progesterone production, initially the result of corpus luteum rescue by hCG and later from a growing placenta, as well as the potential negative influence of hCG itself. The level of aromatase activity necessary to support progressive follicular growth would be difficult to maintain under the combined influence of progesterone and hCG even without the added impact of higher circulating concentrations of androstenedione and testosterone, which rise approximately 2-3-fold during pregnancy. In addition, the negative feedback of progesterone, together with placental estrogen, very effectively reduces the level of gonadotropin stimulation of renewed follicular activity. This central action of luteal and, subsequently, placental steroids provides further insurance, perhaps necessary, against continued follicular development. Indeed, ovulation can be successfully induced with exogenous gonadotropins during pregnancy.[146] The feedback inhibition of gonadotropin secretion provided by placental steroids may assume greater importance as pregnancy progresses and hCG levels decline from the peak concentrations reached at about 10 weeks of gestation.

The corpus luteum of pregnancy eventually regresses in spite of the continued presence of hCG. The point at which the conceptus becomes independent of luteal function varies among species. Luteectomy usually induces abortion in women if carried out before 7 weeks' gestation.[147] In contrast, the fetal rhesus monkey becomes independent of luteal function as early as 6 days after implantation, and always by 3 weeks of gestation.[148] The monkey corpus luteum declines at a time when CG levels have risen to their zenith, ceasing to secrete significant amounts of progesterone, although it continues to produce estradiol.[148,149] Thereafter, the corpus luteum becomes refractory to the tropic effect of hCG, which is possibly the result of prolonged exposure to high concentrations and down-regulation of LH/hCG receptors.[88]

Prolactin and Luteal Function

After a transient fall at the time of luteal demise, progesterone concentrations steadily rise throughout human pregnancy to plateau at 36 weeks' gestation until delivery.[150] The levels of progesterone fall rapidly in the postpartum period but decline more gradually in the nursing mother. Specific binding sites for PRL are present in luteal tissue, but their significance remains unclear (Fig. 6).[151] Whereas most investigators have not found a consistent cyclic change in PRL concentrations during the cycle, some have observed luteal levels slightly higher than those in the follicular phase.[46,152,153] Although PRL may act as an important luteotropic agent in lower mammals, the presence of PRL fails to influence progesterone secretion by human luteal cells maintained in culture and stimulates only a transient increase in the progesterone production of tissue obtained postpartum.[154] There is some in vitro evidence that PRL, at physiologic concentrations, may play a permissive or mildly tropic role in support of luteal function. Progesterone produced by human granulosa cells main-

tained in culture is significantly reduced when PRL present in the culture medium is neutralized with a specific antiserum.[155] The combined administration of bromocriptine and estradiol can induce luteolysis in the monkey when the same dose of either alone has no effect.[156] The same effect has been demonstrated in women.[157] The addition of bromocriptine to a luteolytic dose of estradiol enhances the effect, and continuous administration of bromocriptine throughout the cycle may reduce luteal progesterone production.[158] In contrast, when present in higher concentrations, PRL may inhibit progesterone synthesis.[155] However, ovulatory menstrual cycles induced with pulsatile GnRH replacement in monkeys with hypothalamic lesions exhibit a normal luteal phase, despite marked elevations in circulating levels of PRL.[52] Therefore, luteal phase defects associated with hyperprolactinemia probably result from a disruption of GnRH and subsequent gonadotropin secretion, rather than from an action on the ovary.

Summary. Normal luteal function requires optimal preovulatory follicular development, continued tonic LH secretion, a ready supply of LDL-cholesterol as the obligatory substrate of progesterone production, and perhaps the tropic action of physiologic concentrations of PRL. Progesterone appears to effectively suppress new follicular growth during the luteal phase through an action on the ovary, quite likely complemented by its negative feedback modulation of gonadotropin secretion or enhancement of that of luteal estrogen production. The mechanism of luteolysis in the normal cycle may involve an interruption of the tropic action of LH. The action may take place within the ovary and result from inhibition of LH-binding or prostaglandin-mediated interference with postreceptor events. Alternatively, or, in addition, regression of the corpus luteum may follow a withdrawal of LH stimulation, induced by the feedback inhibition of its own estradiol and progesterone production on gonadotropin secretion. Luteal estrogen production, whether or not involved in the initiation of the luteolytic process at the ovarian or central level, may function to effectively replenish the endometrial progesterone receptors necessary to provide the endometrium with the capacity for continued response to the progesterone produced and to mediate normal secretory development. The otherwise inevitable demise of the corpus luteum is prevented in the fertile cycle by the action of hCG. By perhaps interfering with local prostaglandin synthesis, hCG effects a timely rescue of the corpus luteum, serving to stimulate continued progesterone production and thus maintain luteal function until placental steroidogenesis is well established. Through its elaboration of hCG, the conceptus directs the suppression of any new follicular growth. Effective inhibition of renewed folliculogenesis is assured as hCG teams with progesterone to create an unfavorable intraovarian environment while placental steroids exert negative feedback on gonadotropin secretion. At the height of hCG production, the corpus luteum becomes refractory to further stimulation, and its eventual regression is perhaps the result of down-regulation of LH/hCG receptors and functional deprivation of any tropic support.

The Neuroendocrine Control of Gonadotropin Secretion

From the foregoing discussion it seems evident that gonadal steroids produced during both the follicular and luteal phase act through feedback mechanisms to coordinate gonadotropin secretion with events in the ovary. An understanding of the mechanism through which estrogen and progesterone exert such feedback modulation requires a knowledge of hypothalamic-pituitary interactions involved in gonadotropin release.

Highly specialized neurons in the hypothalamus synthesize and secrete GnRH in response to stimuli both blood-borne and from within the brain. Such neurosecretory cells serve to integrate neuronal input from higher centers with feedback signals from the developing follicle. GnRH is transported

down the axon to its terminal in the region of the median eminence. There it is secreted into the capillary venous network that bathes the anterior pituitary. GnRH binds to a specific membrane receptor present only on the gonadotrope and, through a mechanism involving the movement of calcium ion, stimulates gonadotropin release (Fig. 7).[159]

Immunocytochemical studies have identified neurons containing GnRH throughout the hypothalamus with axons projecting to the median eminence, posterior pituitary, and limbic system. The highest concentration of GnRH neurons occurs within the arcuate nucleus in the medial basal hypothalamus.[160] Isolation of the medial basal hypothalamus from higher centers through stereotaxic deafferentiation has no effect on cyclic gonadotropin release or ovulatory function, whereas radiofrequency lesions in the area of the arcuate nucleus result in a prompt fall of gonadotropins to undetectable levels.[1,52] It appears that the arcuate nucleus is the primary structure mediating the hypothalamic control of gonadotropin secretion. The function of GnRH neurons residing outside this area remains unclear.

Frequent sampling techniques have demonstrated that gonadotropins are released in rapid, rhythmic pulses, superimposed on a low level of continuous secretion.[161] The weight of current evidence suggests that the pulsatile secretion of gonadotropins is not intrinsic to the pituitary but is rather a response reflecting intermittent hypothalamic stimulation.[162] The ovariectomized monkey exhibits a characteristic hourly (circhoral) pattern of pulsatile LH secretion. This physiologic rhythm can be reproduced in castrated animals bearing hypothalamic lesions that eliminate endogenous GnRH secretion. The pulsatile administration of GnRH at hourly intervals restores circhoral LH secretion and returns gonadotropins to preexisting castrated levels. In contrast, a

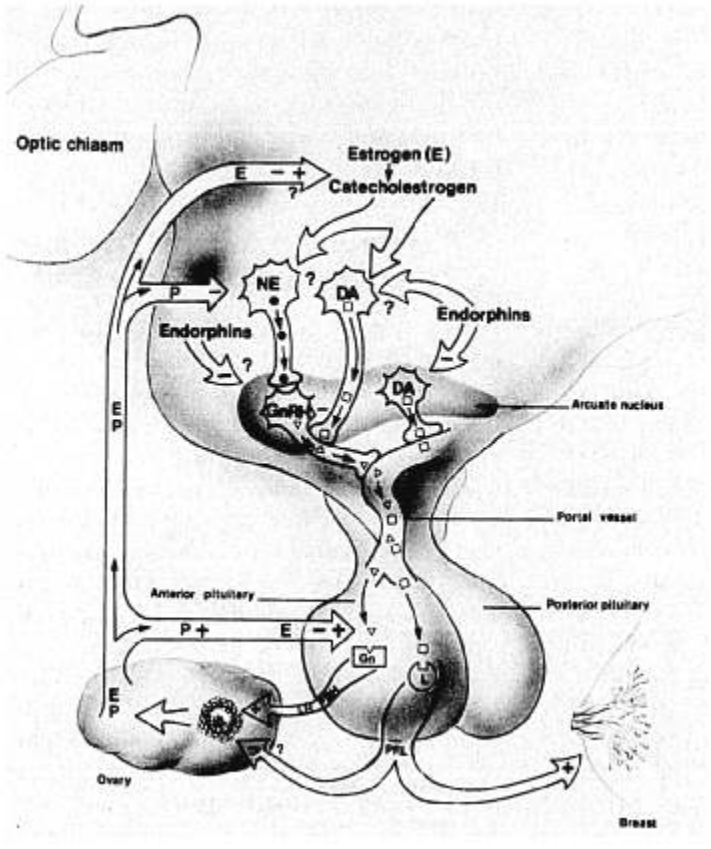

FIG. 7. *The neuroendocrine control of gonadotropin secretion. (From Fritz and Speroff.[1a] By permission.)*

constant GnRH infusion prompts only a transient rise in LH levels which again fall to undetectable levels. More direct evidence has recently been provided by the results of studies using a "push-pull" canula implanted in the area of the median eminence of sheep.[163] The release of GnRH, measured in hypothalamic perfusates, does occur in discrete pulses which preceed or accompany LH pulses of an amplitude that is highly correlated with that of the corresponding pulse of GnRH. Thus, the normal pattern of episodic gonadotropin secretion apparently occurs in response to pulsatile release of GnRH into the portal circulation. Indeed, the direct measurement of GnRH in the portal plasma of pituitary stalk-sectioned rhesus monkeys has clearly demonstrated episodic fluctuations of the releasing hormone.[164] In addition, circhoral pulses of GnRH have recently been detected in the peripheral plasma of women.[165]

The available evidence points to GnRH as a common releasing hormone, capable of stimulating both LH and FSH release.[166] The administration of either a purified hypothalamic extract or synthetic GnRH stimulates the release of both gonadotropins. Variation in the pattern of LH and FSH release is the result of the feedback modulation of gonadal steroids. Within the well-established monthly pattern, the gonadotropins are secreted in a pulsatile fashion with a frequency and magnitude that varies with the phase of the cycle.[167] Pulsatile increments in gonadotropin release occur every 60–90 minutes throughout most of the cycle but decrease in frequency to every 3–4 hours during the mid and late luteal phase. Pulse amplitude is greatest during the midcycle surge and least in the late follicular phase. Increasing estrogen production from the preovulatory follicle may signal the decline in pulse amplitude observed in the late follicular phase. The infusion of exogenous estradiol produces a similar effect.[168] The feedback modulation of elevated progesterone levels may be implicated in the reduction in pulse frequency noted during the luteal phase.

The Site and Mechanism of Gonadal Steroid Feedback

Determination of the sites where estradiol and progesterone exert their feedback effects has been the focus of a great deal of investigative effort and remains controversial. Evidence has been presented to support actions at both the hypothalamic and pituitary levels.

If gonadotropin secretion is reestablished in castrated monkeys bearing hypothalamic lesions by a pulsatile infusion of GnRH, both the positive and the negative feedback effects of estradiol administration remain unaffected.[169,170] These findings suggest that the feedback modulation of estradiol occurs at the level of the pituitary gland. Corroborative evidence has come from experiments performed in animals after transection of the pituitary stalk.[171] Estrogen-induced gonadotropin surges are observed before and after stalk-section and placement of a Silastic barrier between the severed ends. When pulsatile GnRH replacement is provided for animals with arcuate lesions with intact ovaries, normal ovulatory menstrual cycles can be induced.[172] As a result, it has been suggested that GnRH plays only a permissive, although obligatory, role in the control of gonadotropin secretion and that feedback modulation of gonadal steroids, acting directly on the pituitary, produces the pattern of gonadotropin secretion observed in the menstrual cycle.[172]

It does seem clear that estradiol can modulate gonadotropin secretion through an action at the level of the pituitary gonadotrope. Autoradiographic studies have demonstrated estrogen receptors in the anterior pituitary.[173] Pituitary responsiveness to GnRH is dependent on the duration of estrogen exposure and proportional to the circulating concentration of estradiol.[174] When exposed to increasing levels of estradiol in a manner similar to that observed during the late follicular phase, the pituitary responds to the GnRH challenge with a prolonged and augmented pattern of gonadotropin release.[175] In contrast, short-term exposure to preovulatory levels of estradiol

actually blunts the pituitary response to a bolus of GnRH when compared with that of unprimed controls.[176] Low levels of estradiol have no effect on pituitary sensitivity to GnRH, whereas the same duration of exposure to higher concentrations augments GnRH-induced gonadotropin release.[56]

Whereas the positive feedback of estrogen has been generally regarded as mediated through a direct action on the pituitary, its inhibitory effects on gonadotropin release may be exerted at both hypothalamic and anterior pituitary levels. The microinfusion of estradiol into the third ventricle or anterior pituitary appears to inhibit gonadotropin release through different mechanisms.[177] When delivered directly to the pituitary, estradiol decreased responsiveness to GnRH stimulation. When infused into the central nervous system, a decline in the neurosecretion of GnRH was suggested.

The feedback actions of progesterone may also be exerted at sites within both the hypothalamus and the pituitary gland (Fig. 7). A typical gonadotropin surge, induced by a bolus of estrogen during the follicular phase, does not occur in the presence of progesterone at luteal phase concentrations.[66] However, an estradiol-induced gonadotropin surge does occur in monkeys with arcuate lesions on pulsatile GnRH replacement whether or not progesterone is present.[178] Thus, progesterone would appear to exert its negative feedback at the hypothalamic level and block the estrogen-induced positive feedback response through interference with GnRH release. It is difficult to interpret the finding that estradiol-induced gonadotropin release is not observed in the presence of progesterone when a pulsatile GnRH infusion is imposed on intact but "acyclic" animals.[179] The manner in which an exogenous pulsatile GnRH infusion, superimposed on some level of endogenous rhythm, might influence the pituitary and its response in any given steroid environment is open to speculation. In contrast to the failure of progesterone to inhibit estradiol-induced gonadotropin release in the arcuate-lesioned, GnRH-replaced animal, the ability of progesterone to augment and advance the gonadotropin surge in response to estrogen, when administered after estradiol priming, remains intact.[178] This suggests that progesterone may act to facilitate the midcycle surge at the pituitary level. However, it was recently demonstrated that the ability of progesterone to induce an LH surge in the presence of subthreshold levels of estradiol can be effectively blocked with pentobarbitone anesthesia, a result implicating mediation through higher centers in the brain.[180]

It has been proposed that estradiol may modulate pituitary sensitivity to GnRH and subsequent gonadotropin secretion by altering the GnRH receptor content of the gonadotrope.[52] However, pituitary responsiveness to GnRH does not always reflect its tissue receptor concentration.[181,182] During estrogen priming, GnRH-induced LH release is initially suppressed, despite the fact that pituitary GnRH receptor content rises steadily with increasing duration of estrogen exposure. Pituitary responsiveness to a GnRH challenge is positively correlated with receptor concentrations only when positive estrogen feedback is in effect. The findings suggest that whereas the positive feedback mechanism may involve an increase in GnRH receptor concentration, the negative component of estrogen feedback effects operates through a different mechanism.[183]

Whether or not any hormonally induced changes in GnRH receptor concentrations are the result of direct effects of gonadal steroids on the pituitary or are mediated via the hypothalamus is not yet clear. The positive feedback effect of estrogen, although positively correlated with an increase in the level of pituitary GnRH receptors, may not necessarily be the result of a direct action on the pituitary itself. It is becoming clear that GnRH can regulate the concentration of its own pituitary receptor. GnRH can self-prime the response of bovine pituitary cells in culture and thereby increase the quantity of gonadotropins released by a subsequent GnRH exposure.[184] The degree of the effect is dependent on the dose and number of

GnRH exposures and the ovarian steroid milieu. Administered alone, estrogen enhances GnRH priming of its own receptor. In contrast, progesterone inhibits the effect when introduced alone or in combination with estradiol.[184] Endogenous GnRH secretion is essential for the maintenance of pituitary GnRH receptor levels and subsequent gonadotropin secretion.[185] Up-regulation of GnRH receptors in the anterior pituitary occurs in response to low doses of continuous infusion of GnRH or its agonist.[186] The postcastration rise observed in pituitary GnRH receptors is dependent on an increase in GnRH secretion.[187,188] A loss of GnRH receptors is produced by continuous high-dose infusions of GnRH.[186] Indeed, GnRH can induce an acute down-regulation of its own pituitary receptor.[182,189] Such an abrupt fall in pituitary GnRH receptor content occurs coincident with the preovulatory surge of gonadotropins and there is evidence suggesting that there is a preovulatory rise of GnRH in portal blood.[183,190] A periovulatory rise in GnRH may even be detectable in the peripheral plasma.[165]

If estrogen exerted its feedback effects at a hypothalamic level rather than, or in addition to, a direct action on the pituitary, the observed response in gonadotropin secretion could also, in part, result from its alteration of GnRH release and, in turn, the influence of GnRH on the level of its own pituitary receptor. A reduction in GnRH secretion secondary to the negative feedback of low levels of estrogen might promote the increase in receptor concentration observed during estradiol priming. Further, the positive feedback of estrogen, exerted with sustained higher levels, may involve an acute GnRH discharge magnified by then peak receptor concentrations. Such an acute release of GnRH may also induce the down-regulation of GnRH receptors and account for the rapid decline in receptor concentration observed at midcycle.[191] This mechanism would necessarily imply that the hypothalamus plays a much more active role than has previously been suggested.

The conclusion that the hypothalamus exerts a necessary but only passive influence on gonadotropin secretion has been based on the assumption that, in the experimental designs employed, any hypothalamic input was eliminated (pituitary stalk-section) or bypassed (arcuate nucleus lesion and GnRH replacement), or both. Recent evidence has led to speculation regarding the completeness of the separation of the hypothalamus and pituitary in previous studies. The result of pituitary stalk-section and pulsatile GnRH replacement in monkeys with intact ovaries differs with the nature of the barrier interposed between the severed ends.[192] Animals receiving a Teflon barrier failed to ovulate although gonadotropin secretion was reestablished and preovulatory levels of estradiol were attained. Animals treated in the same fashion but provided with Silastic barriers experienced a return of ovulatory function. The striking dissimilarity of response suggests that a "specific hypothalamic message," quite possibly an acute release of GnRH, may indeed be required for the preovulatory gonadotropin surge.[192]

Thus, the gonadal steroids may exert their feedback effects on gonadotropin secretion, at least in part, by modulating the magnitude and frequency of GnRH secretion (Fig. 7).[193] In fact, the significance of alterations in the frequency and amplitude of pulsatile GnRH has recently been demonstrated.[194] In ovariectomized monkeys with arcuate lesions, increasing the frequency of pulsatile GnRH replacement to more than the normal frequency, once per hour, results in a progressively reduced pituitary response and a gradual decline in gonadotropin levels. Decreasing the frequency of GnRH pulses alters the pattern of gonadotropin secretion as LH levels decline and FSH rises. A reduction in the amplitude of hourly GnRH pulses suppresses the release of both gonadotropins, whereas increasing the magnitude of each hourly pulse preferentially reduces FSH secretion.[194] These observations suggest that virtually any pattern of gonadotropin secretion could be induced, and may thus result, from alterations in

GnRH pulse amplitude and frequency that may, in turn, reflect the hypothalamic feedback of the gonadal steroids.

Another intriguing aspect of the pattern of gonadotropin secretion deserves mention. There is a growing recognition of the heterogeneity of the pituitary gonadotropins. Both FSH and LH may be secreted in different forms with a corresponding variation in the relative bioactivity of the molecule. The functional significance of this phenomenon is illustrated by the marked disparity that exists between the patterns of LH secretion during the midcycle gonadotropin surge as determined by radioimmunoassay and bioassay.[195,196] The pattern of bioactive LH is temporally and quantitatively distinct from the pattern observed in radioimmunoassayable LH and suggests that the LH released at midcycle may be a more biologically active molecule than that secreted at other times in the cycle.[196] Such qualitative differences in the LH molecule have been observed in women. The differing biologic activity observed in samples obtained at midcycle, postmenopause, after the administration of exogenous GnRH, and in findings implicating the influence of the sex steroid environment.[197] Earlier studies demonstrated that FSH was also released in at least two forms. FSH extracted from the pituitaries of ovariectomized monkeys has a higher molecular weight, a slower metabolic clearance rate, and twice the biologic activity of that derived from intact animals, although both forms do coexist.[198] More recently, no less than six different species of FSH were identified in the hamster pituitary.[199] There is some evidence that, like LH, the FSH released during the gonadotropin surge may have enhanced biologic activity.[200]

Whereas immunologic activity appears to reside primarily in the protein backbone of the glycoprotein hormones, biologic activity may be determined by the carbohydrate component.[197] The FSH of women of reproductive age is less acidic than that of men or postmenopausal women.[201] The differences appear to reflect variation in the sialic acid content of the molecule. There is a well-established relationship between the activity and half-life of glycoprotein hormones and their sialic acid content.[202,203] Incorporation of sialic acid residues into the hormone appears to play a critical role in the observed structural heterogeneity and may be the result of the influence of sex steroids or even GnRH on enzymatic processes within the gonadotrope.[199] Indeed, treatment with estradiol can induce less acidic forms of FSH in the sera of men.[201] Thus, the feedback effects of gonadal steroids may include modulation of sialylation and the subsequent size and activity of the gonadotropins released.[196,198] The effect appears to be most evident in hypergonadotropic states.

In addition to the apparent enhanced bioactivity of the LH secreted at midcycle, a more biologically potent form of LH has recently been demonstrated in a group of patients with polycystic ovaries, which may, in part, explain the elevated androgen levels observed in such patients.[204] Further study of the bioactivity of gonadotropins may provide greater insight into both normal physiologic mechanisms and the pathophysiology of endocrinopathies.

Neurotransmitters

GnRH is secreted from hypothalamic neurosecretory neurons centered in the arcuate nucleus with axons projecting to the median eminence. Its release occurs in response to diverse neural inputs operating through neurotransmitters. Researchers believe that the catecholamines may be the principal neurotransmitters involved in GnRH secretion, although several small peptides have also been implicated.[205]

A stimulatory role for norepinephrine in the control of gonadotropin secretion has been reasonably well-established. Alpha-adrenergic blocking agents, administered to ovariectomized monkeys, inhibit pulsatile gonadotropin secretion. In contrast, β-adrenergic blockade is without effect.[1] However, the inhibitory action of α-blockade is not observed in the animal with an arcuate lesion on GnRH replacement.[170] Further,

the selective depletion of hypothalamic norepinephrine eliminates pulsatile LH secretion in ovarectomized rats.[206] These studies, among others, suggest that norepinephrine acts as an excitatory neurotransmitter in the modulation of GnRH release.[207,208] Indeed, it was recently demonstrated that norepinephrine stimulates release of GnRH from terminals in the median eminence by first interacting with an α-adrenergic receptor.[209] As LH levels start to rise following ovariectomy in the rat, the increase is associated with a peak in the norepinephrine content of the suprachiasmatic, medial preoptic region and a sharp drop in the dopamine and GnRH present in the median eminence.[210] As LH levels reach their peak, norepinephrine in the preoptic region returns to lower levels, whereas dopamine and GnRH increase in the media eminence.[210] These observations suggest that both norepinephrine and dopamine are involved in the pulsatile release of LH (Fig. 7).

Dopamine, the immediate precursor to norepinephrine in catecholamine synthesis, inhibits the release of both gonadotropins and PRL.[211-214] It appears to act directly on the lactotrope to inhibit PRL release. At physiologic concentrations, dopamine causes a prompt inhibition of cAMP accumulation in cultured anterior pituitary cells which may be part of the mechanism underlying dopaminergic inhibition of PRL release.[215] A great deal of evidence is accumulating to indicate that dopamine is, in fact, the PRL-inhibiting factor that maintains normal tonic inhibition of PRL secretion.[216-218] Furthermore, since human prolactinoma cells possess dopamine receptors and exhibit a dose-related sensitivity to the suppression of PRL secretion by dopamine, it has been suggested that PRL-secreting pituitary adenomas arise from lactotropes which are deprived of dopamine inhibition.[219] It is hypothesized that prolactinomas are the result of a hypothalamic defect in dopamine production or the interruption or occlusion of portal flow to a selected region of the pituitary which is then revascularized from accessory vessels.[219] There is recent evidence that animals with estradiol-induced—PRL-secreting tumors may similarly suffer from a defect in dopamine neurotransmission.[220]

In addition to suppressing the pituitary lactotrope via its direct neurosecretion into the portal circulation, dopamine appears to inhibit gonadotropin release through a central action on the GnRH neuron. Immunohistochemical studies have identified dopaminergic neurons in close contact with GnRH nerve terminals in the medial eminence.[221] Rather than synapsing with the GnRH cell body, dopaminergic neurons may exert their influence on LH secretion through "axo-axonal" communications in the area of the median eminence.[222] In this relationship, GnRH secretion will reflect a balance of noradrenergic excitation and dopaminergic inhibition (Fig. 7). Dopamine suppression of gonadotropin release is most marked in the preovulatory period. This selective hypersensitivity, at a time when LH levels are highest and, presumably, GnRH activity may be increased, suggests that dopamine acts by inhibiting GnRH release.[213] In addition, the findings suggest another potential mechanism for central estrogen feedback modulation.

Estrogen receptors appear to exist in the cell bodies of arcuate dopaminergic neurons.[223] Estradiol injections increase dopamine neuronal activity and decrease noradrenergic activity in the median eminence.[224] Studies in ovariectomized women have demonstrated that estrogen can modify dopamine's inhibition of gonadotropin secretion.[222] Together, these studies indicate that an element of estrogen feedback effects may involve modulation of the inhibitory influence of dopaminergic neurons on GnRH release.

A similar mechanism may be involved in the well-known inverse relationship between PRL and gonadotropin secretion. PRL stimulates dopamine neuronal activity in the median eminence.[224] The infusion of dopamine reduces serum LH but not FSH when administered to both normal and

hyperprolactinemic women.[22] Similarly, the response of pituitaries from lactating rats to a GnRH stimulus reflects a suppression of basal LH but not FSH secretion, suggesting the earlier influence of dopamine in vivo.[225] When pituitary stalk-sectioned, castrated monkeys are provided with pulsatile GnRH replacement neither the induced hyperprolactinemia nor the subsequent infusion of dopamine alters pulsatile LH release.[226] These studies suggest a mechanism whereby elevated PRL levels may result in acyclic gonadotropin release. Hyperprolactinemia may stimulate the release of dopamine through a short-loop feedback mechanism. The resulting increase in dopaminergic inhibition may then alter the pattern of GnRH secretion, leading to a reversed LH/FSH ratio, anovulation, and amenorrhea. Interestingly, as discussed earlier, a decrease in the frequency of GnRH pulses can induce the same pattern of disparate gonadotropin release in experimental animals.[194] The oligomenorrhea commonly observed in women involved in endurance running programs or similarly strenuous physical conditioning may be mediated through such a mechanism. As weekly mileage increased, PRL responses stimulated by thyroid-stimulating hormone-releasing factor progressively increased as well, suggesting that one mechanism responsible for menstrual dysfunction in such individuals may be frequent or exaggerated PRL responses to exercise.[227]

In addition to norepinephrine and dopamine, several other apparent neurotransmitters may be involved in the modulation of gonadotropin secretion. Serotonin and the pineal indole, melatonin, appear to inhibit gonadotropin release, whereas gamma aminobutyric acid and acetylcholine have been reported to have the opposite action.[205,228,229] Certain peptides found in the brain have also been implicated. Of these, the endorphins are of particular interest.

Endorphins

It is now evident that ACTH shares a common precursor molecule with another pituitary peptide known as β-lipotropin. Additionally, both ACTH and β-lipotropin can serve as precursors for other biologically active peptides. Cleavage products of β-lipotropin include a class of smaller peptides with morphinelike activity, the endorphins (Fig. 8).[230] Increasing evidence suggests that endorphins are involved in the regulation of hypothalamic-pituitary hormone secretion.

The finding that both morphine and β-endorphin stimulate PRL release in intact but not pituitary stalk-sectioned monkeys suggested a hypothalamic site of action.[231,232] The localization of opiate receptors on dopaminergic neurons,[233] and the demonstration that endorphins inhibit the release of dopamine into the portal blood suggested that opiates may raise PRL levels by decreasing the tonic inhibition of PRL release (Fig. 7).[234]

The endorphins also appear to be involved in the modulation of gonadotropin secretion. The gonadotropin response to the administration of an opiate receptor antagonist varies with the phase of the cycle.[235] The infusion of naloxone during the luteal but not the early follicular phase raises LH levels. This rise is the result of an increase in both the frequency and amplitude of pulsatile LH secretion.[236] The effect correlates well with the levels of β-endorphin in the portal blood of the rhesus monkey during the cycle. Concentrations of β-endorphin

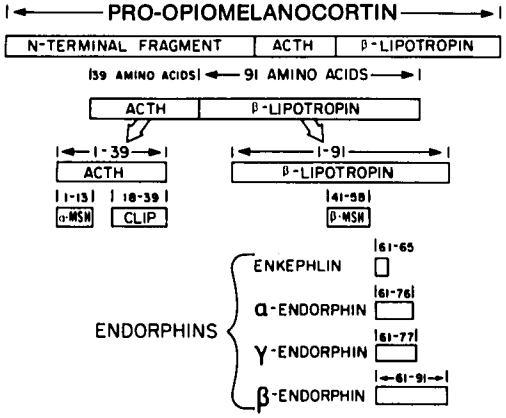

FIG. 8. *The derivation of endorphins. (From Fritz and Speroff.[1a] By permission.)*

are highest during the luteal phase and the latter half of the follicular phase and undetectable at menses.[237] The results suggest that endorphins may participate in the feedback regulation of gonadotropin secretion. This possibility was lent further support when the effect of exogenous steroids on portal blood endorphin levels was examined.[238] When administered to ovariectomized monkeys, estradiol had no consistent effect on portal endorphin concentrations. However, the combined administration of both estradiol and progesterone induced marked elevations in the levels of β-endorphin in portal blood. Although the secretion of β-endorphin from the hypothalamus directly into hypophyseal portal blood suggests a direct opiate effect on the gonadotrope, the LH response to a fixed GnRH stimulus in stalk-sectioned monkeys is not prevented by morphine.[239,240] In addition, morphine does not prevent an estradiol-induced LH surge when both are administered early in follicular phase.[240] Furthermore, opiates have no effect on basal or GnRH-induced LH release by pituitaries in culture.[241] Thus, cyclic changes in the sex steroid environment may affect anterior pituitary function, in part, through hypothalamic endorphin action and synthesis. Indeed, the observation that portal endorphin concentrations are highest in the luteal phase and can be elevated by the combined administration of estradiol and progesterone implicates the action of endogenous opiates in the reduced pulse frequency of gonadotropin secretion during the luteal phase. These studies suggest that endogenous opiate peptides may directly inhibit GnRH neuronal activity, operate indirectly through suppression of noradrenergic neurons, or modulate GnRH release at the level of the median eminence (Fig. 7).

Alternatively, endorphins may affect gonadotropin secretion through an action on dopaminergic neurons (Fig. 7). Direct intravenous injection of β-endorphin during the early follicular phase promptly elevates PRL levels and brings about an eventual decline in LH concentrations.[242] Similarly, the administration of morphine to postmenopausal women effects a prompt increase in PRL levels, accompanied by a significant reduction in LH concentrations.[243] The eventual decline in LH levels observed after β-endorphin injection is preceded by a transient rise. The decline occurs coincident with the peak in PRL concentrations.[242] A reduction in dopaminergic activity might initially withdraw inhibition of GnRH secretion while stimulating PRL secretion, prompting a rebound increase in dopamine release and suppression of GnRH. Such a mechanism could explain the biphasic LH response.

Regardless of the mode of action, the potential for adverse effects on menstrual function is apparent. Increased endogenous opiate activity has been implicated in hypothalamic amenorrhea and suggested as the cause of the suppressed gonadotropins and elevated PRL levels observed in association with stress and exercise.[224,245]

Catecholestrogens

Appreciation of the roles of catecholaminergic neurotransmitters has combined with accumulating evidence for a central estrogen feedback action to stimulate interest in a potential mechanism for their interaction. Investigation of estradiol metabolism in the brain has revealed that the hypothalamus is rich in 2-hydroxylation activity.[246] The enzymatic addition of a hydroxyl (−OH) group at this position gives estrogen remarkable structural similarity to the neurotransmitters, norepinephrine and dopamine (Fig. 9). As a result, it is perhaps not surprising that the enzyme responsible for the degradation of these catecholamines also metabolizes the catecholestrogens.[247] However, catechol-O-methyl transferase (COMT) actually exhibits a greater affinity for the cholecholestrogens than for catecholamines. As a preferred substrate, the catecholestrogens may effectively compete for hypothalamic COMT and thus have the capacity to alter the effective concentrations of neurotransmitters.

By inducing a transient elevation of the

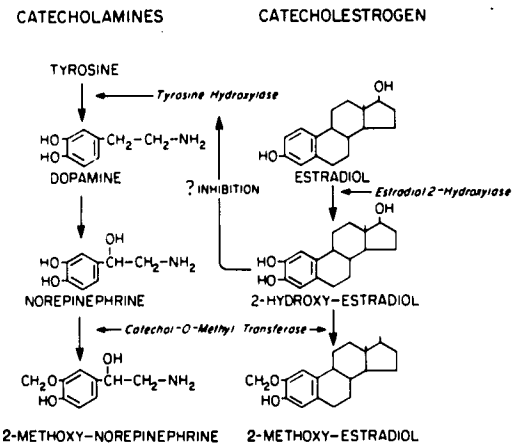

FIG. 9. *Catecholestrogens. (From Fritz and Speroff.*[1a] *By permission.)*

catecholamine content of the hypothalamus, the catecholestrogens could influence GnRH neuronal activity and modulate gonadotropin secretion. In contrast, by inhibiting tyrosine hydroxylase, the rate-limiting step in catecholamine synthesis, the catecholestrogens may also effectively reduce hypothalamic levels of the neurotransmitter (Fig. 9).[248] Alternatively, they may exert feedback actions directly through estrogen receptor mechanisms or interact with catecholamine receptor sites.[249-251]

The unique structural characteristics of the catecholestrogens make them attractive candidates for an intermediary role in the feedback modulation of both gonadotropin and PRL secretion. Indeed, the administration of catecholestrogens (2-OH estrone and 2-OH estradiol) has been reported to result in both negative and positive feedback effects on gonadotropin release as well as a rise or fall in PRL.[252-255] Clearly, further investigation is warranted in this promising new area.

Summary. The gonadotropins are secreted in a pulsatile fashion in response to the similar pulsatile release of GnRH from neurosecretory neurons centered in the arcuate nucleus of the medial basal hypothalamus. The pattern of pulsatile gonadotropin secretion varies with the phase of the cycle, altered by the feedback modulation of the gonadal steroids. Available evidence suggests that estradiol and progesterone exert their feedback effects both directly on the pituitary and through modulation of the pulsatile pattern of GnRH release. Alteration of the GnRH pulse frequency and amplitude can produce any number of secretory patterns in the relative amounts of FSH and LH released. The feedback influence of gonadal steroids may also involve regulation of gonadotrope enzymes responsible for the incorporation of sialic acid residues in the gonadotropin molecule which, in turn, determines their size and subsequent biologic activity. GnRH release is under the control of catecholaminergic neurotransmitters. Norepinephrine appears to act as an excitatory agent, whereas dopamine inhibits GnRH secretion. Dopamine also directly inhibits PRL release and is probably the PRL-inhibiting factor. The endorphins are endogenous opiate peptides, derived from a common ACTH/β-lipotropin precursor molecule synthesized in the hypothalamus. Through modulation of neurotransmitter mechanisms, direct actions on the GnRH neuron, or stimulation of PRL release, the endorphins may also have impact on gonadotropin secretion. The catecholestrogens, by virtue of their structural similarity to the neurotransmitters, may be involved in mediating the central feedback actions of the gonadal steroids.

Summary

Obviously, the endocrine mechanisms involved in producing the normal, cyclic pattern of menstrual bleeding are exceedingly complex. A review of even our current, far from complete, knowledge of the regulation of follicular growth, cyclic selection of a single dominant follicle, ovulation, and the neuroendocrine control of all three mechanisms only serves to emphasize the myriad of endogenous and exogenous factors that may adversely affect such a delicate balance and be manifest in menstrual disturbance. Indeed, one may wonder that the menstrual cycle is cyclic and predictable at all. Nevertheless, the efficiency with which the system normally operates is striking. Its very com-

plexity often makes disorders of menstrual function a not infrequent symptom of disease outside the reproductive tract, a fact that should stress the need for prompt and thorough evaluation.

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