3 The central control of follicular maturation and ovulation in the human

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I INTRODUCTION

The development of radioimmunoassays has made it possible to describe in detail the hormonal changes both during the normal menstrual cycle and during the experimental manipulations that have been performed to unravel its regulatory mechanisms. With the synthesis of gonadotrophin-releasing hormone (GnRH), an important tool became available to promote further research, especially in the field of the central regulation

of gonadotrophin secretion. Studies of the neural control of the primate menstrual cycle, published a decade ago, have resulted in the formulation of the concept of the solely permissive, albeit obligatory, role of hypothalamic GnRH in the regulation of the menstrual cycle, with the ovarian feedback taking place at the level of the pituitary gland. These studies laid the physiological basis for a new therapeutic approach, the unvariant, low-dose, pulsatile administration of GnRH in hypothalamic amenorrhoea.

At the time of the formulation of this concept, however, it was already evident (by inference) that hypothalamic GnRH secretion changes during the menstrual cycle and that these alterations in hypothalamic GnRH activity were imposed upon the hypothalamus by functional changes of the ovary during the course of the cycle. Recent studies have revealed the mediating role of the hypothalamic endogenous opioid system in this respect. There is a still-ongoing debate, with diverging views in the literature, on the neuroendocrine regulation of the human menstrual cycle, especially concerning the involvement of the hypothalamic GnRH pulse generator in the ovarian feedback regulation, and its functional significance.

In this review, we attempt to summarize recent data on the central regulation of the human menstrual cycle, and to propose a model of the function of the hypothalamic–pituitary–ovarian axis that incorporates novel findings and integrates formerly divergent concepts. Intraovarian regulatory aspects of follicular maturation will only be reviewed when necessary.

II HISTORICAL OVERVIEW

Since the beginning of the century, when it was shown that hypophysectomy and hypothalamic lesions resulted in gonadal atrophy (Aschner 1912), a central control over ovarian function has been postulated. More than 50 years ago, Hohlweg and Junkmann (1932) and Hohlweg (1934) formulated the concept of a dual control of gonadotrophic pituitary function exerted by both the hypothalamus and the ovaries. Later, it was demonstrated that the neural structures within the hypothalamus that are essential for the maintenance of anterior pituitary function are located within basal parts of the hypothalamus, termed the hypophysiotropic area (Szegentagothai *et al.* 1968). While the concept of the dual control of the pituitary gonadotrophin secretion has been substantiated by further research, especially during the past two decades, the original assumption of a direct neuronal control of the pituitary by the hypothalamus (Hohlweg and Junkmann 1932) had to be abandoned, since the appropriate neuronal connections could not be demonstrated.

It was Harris who recognized the importance of the pituitary portal

system initially described by Popa and Fielding. Harris concluded from his experiments that the hypothalamic control of pituitary function was neurohumoral in nature and mediated by neurohumoral agents that were secreted from the nerve terminals of the median eminence into the portal system (Harris and Naftolin 1970). Following his suggestion, these neurosecretory products were termed releasing or inhibiting factors, and after their identification, releasing or inhibiting hormones (Schally *et al.* 1971). Gonadotrophin-releasing hormone (GnRH) was the second of these neurohumoral agents to be isolated, structurally identified, and synthesized. This was achieved by the groups of Schally and Guillemin in 1971; after this time, the synthetic hormone became available and GnRH has been used extensively as a tool in neuroendocrine research.

Early attempts to use GnRH clinically for the treatment of reproductive disorders that were supposed to be due to an inadequate secretion of endogenous GnRH, however, were of only limited success. Effective therapeutic use had to await further progress in the understanding of the physiological mechanisms that control gonadotrophin secretion and gonadal function. In this respect, the work of Knobil and co-workers was of utmost importance. They first demonstrated a pulsatile secretion of pituitary LH at an hourly (circhoral) frequency and that a pulsatile stimulation of the anterior pituitary by GnRH with this frequency was a prerequisite for normal pituitary function (Dierschke *et al.* 1970; Belchetz *et al.* 1978). Furthermore, on the basis of their experiments involving the destruction of the arcuate nucleus and replacement of endogenous GnRH by pulses of exogenous GnRH unvaried in dose and frequency, they proposed that hypothalamic GnRH has only a permissive, albeit obligatory, role in the regulation of the menstrual cycle, with the ovarian steroid feedback taking place directly at the level of the pituitary gland rather than at the level of the hypothalamus (Nakai *et al.* 1978; Knobil 1980*a*; Knobil *et al.* 1980). The pulsatile administration of GnRH using an unvaried dose and frequency to women suffering from hypothalamic amenorrhoea (a disturbance presumably due to a reduced or absent hypothalamic secretion of GnRH) was the successful clinical application of this physiological concept (Leyendecker 1979; Leyendecker *et al.* 1980*a*, *b*).

Although menstrual cycles that do not differ from normal ones can be induced by the unvaried pulsatile administration of GnRH in GnRH-deficient primates and women, the claim that the pattern of gonadotrophin secretion during the cycle can be described solely by the interaction of GnRH and ovarian steroids on the pituitary did not gain general acceptance (Norman 1983). These diverging views could be explained in part by methodological differences in the respective studies (Norman *et al.* 1983; Plant 1986). There is also accumulating evidence, however, on the

significance of the endogenous opioid system in the regulation of reproduction (Ferin *et al.* 1984; Howlett and Rees 1987) as well as increasing evidence on the significance of the inhibin system in the regulation of follicle-stimulating hormone (FSH) secretion (de Jong 1987). Such evidence renders incomplete any concept on the central regulation of the menstrual cycle that does not include at least these two systems.

III THE CYCLIC CHANGES DURING THE MENSTRUAL CYCLE

The failure of luteal function at the end of an infertile cycle signals the initiation of a new cycle and initiates characteristic functional changes at the levels of the hypothalamus, the pituitary, the ovary, and the endometrium. These functional changes are reflected in the cyclic and pulsatile pattern of gonadotrophin secretion and in the follicular dynamics resulting in the recruitment of a cohort of follicles, the selection and further promotion of the dominant follicle, ovulation, and corpus luteum formation. The ovarian changes are reflected in turn by the pattern of secretion of ovarian hormones (oestradiol, progesterone, and inhibin).

1 The cyclic pattern of hormone secretion

As soon as radioimmunoassays were available, the cyclic pattern of the pituitary gonadotrophins, luteinizing hormone (LH) and FSH, and of the ovarian steroids in blood were described (Midgley and Jaffe 1968; Ross *et al.* 1970; van de Wiele *et al.* 1970; Mishell *et al.* 1971; Abraham *et al.* 1972; Leyendecker *et al.* 1972, 1975*a*; Moghissi *et al.* 1972).

The follicular phase of the cycle begins with slight increases in LH and FSH, as well as a gradual rise in serum oestradiol reflecting follicular growth. During the late follicular phase, serum levels of oestradiol rapidly rise, concomitantly with the development of the dominant follicle, and reach highest values on the day before the midcycle LH and FSH peak.

The preovulatory oestradiol rise comes from the maturing dominant follicle (Baird and Fraser 1975; Channing and Caudert 1976; McNatty *et al.* 1976; Aedo *et al.* 1980; diZerega *et al.* 1980; Goodman and Hodgen 1983). By means of high-resolution ultrasonography, the dominant follicle can be detected easily 6–8 days before the LH peak, when it has reached a diameter of 10–12 mm (Hackeloer *et al.* 1979; Leyendecker *et al.* 1980*b*; Nitschke-Dabelstein *et al.* 1980; Wildt *et al.* 1983). With a rather constant growth rate for the follicular diameter of 1–2 mm day^{-1} (Hackeloer *et al.* 1979), the preovulatory follicle reaches a diameter of 22–24 mm immediately before ovulation. As soon as the dominant follicle can be identified, the smaller accompanying follicles in both ovaries disappear. With the

development of the dominant follicle and its increasing secretion of oestradiol and possibly inhibin, FSH levels decline. The LH levels remain essentially unchanged or even tend to rise (Ross *et al.* 1970; Hoff *et al.* 1983), resulting in the characteristic change in the ratio of LH and FSH concentrations in serum during the midfollicular and late follicular phase (Fig. 3.1).

During midcycle, there is a rapid increase in serum LH and an elevation in FSH that lasts for about 48 h (Thorneycroft *et al.* 1974). The LH surge induces ovulation and the formation of the corpus luteum. The luteal phase is characterized by elevated serum levels of progesterone and oestradiol, and by mean serum levels of both gonadotrophins which are lower than the respective mean levels of the follicular phase (Reame *et al.* 1984; Soules *et al.* 1984). Increased progesterone secretion begins prior to ovulation, as indicated by the rise in progesterone during the LH midcycle surge, and maximal serum levels are reached in the midluteal phase (Leyendecker *et al.* 1972; Thorneycroft *et al.* 1974; Leyendecker *et al.* 1975*a*; Laborde *et al.* 1976; Hoff *et al.* 1983). When the corpus luteum is not stimulated by rising chorionic gonadotrophin from the implanted trophoblast and not transferred into a corpus luteum graviditatis (corpus luteum of pregnancy), its function is maintained for about 14 days. With the regression of the corpus luteum, indicated by the decline in serum progesterone and oestradiol, menstruation ensues and a new cycle begins. The rise of FSH begins prior to menstruation, while the rise in LH is somewhat delayed and occasionally starts even one day after the onset of menstruation (Mais *et al.* 1987; Vermesh and Kletzky 1987).

2 The pulsatile pattern of hormone secretion

As first demonstrated in the rhesus monkey (Dierschke *et al.* 1970), the pituitary release of LH is pulsatile in nature and reflects a pulsatile stimulation of the pituitary gonadotrophs by hypothalamic GnRH (Carmel *et al.* 1976; Clarke and Cummins 1982; Levine *et al.* 1985). These findings were extended to the human (Midgley and Jaffe 1971; Yen *et al.* 1972*b*; Santen and Bardin 1973). Following the demonstration of the physiological and pathophysiological, as well as therapeutic, significance of this pulsatile phenomenon, a new scientific interest emerged and the patterns of pulsatile gonadotrophin secretion during the human menstrual cycle have been extensively characterized. While earlier studies were often based upon sampling intervals that were too long and over sampling periods that were too short, recent studies have used improved sampling regimens in order to obtain reliable results concerning the frequency of LH pulses and, by inference, reliable estimates of the pattern of hypothalamic GnRH activity (Veldhuis *et al.* 1986; Veldhuis 1987).

In spite of the use of a variety of sampling regimens and pulse detection

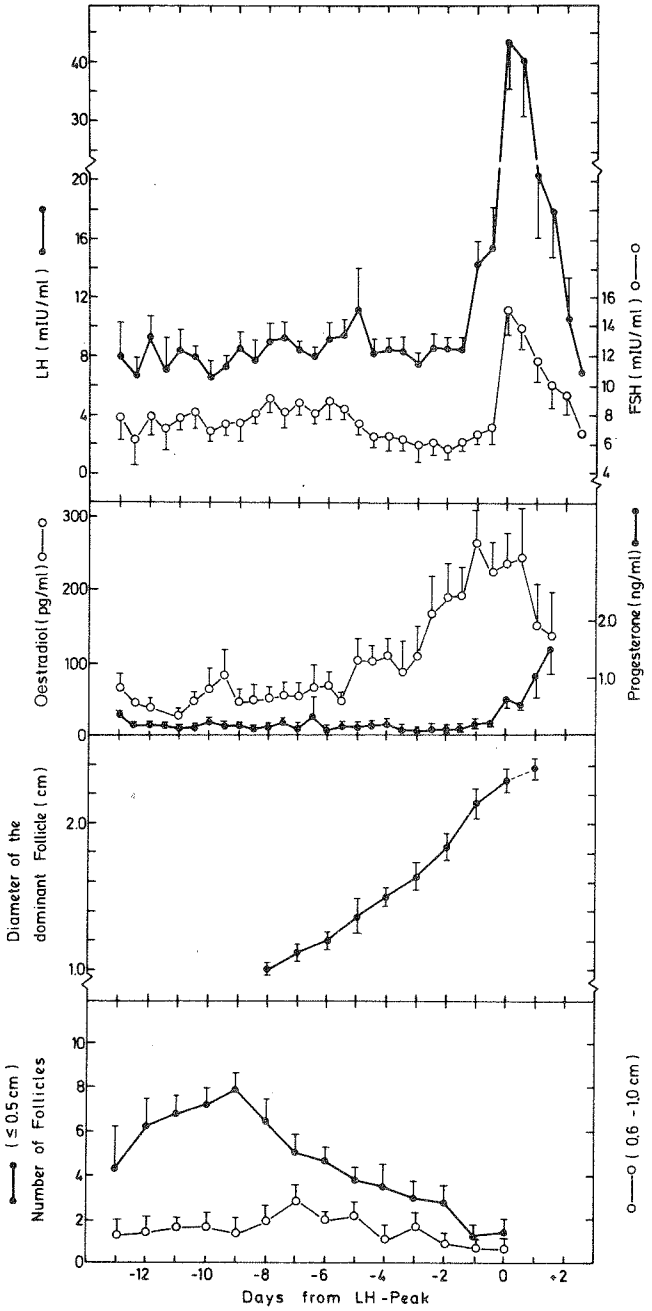

Fig. 3.1 Pattern of LH, FSH, oestradiol, and progesterone secretion, diameter of the dominant follicle, and number of follicles, during the normal menstrual cycle (normalized to the LH surge as day 0). Each point represents mean ± SEM of seven observations. Blood samples were obtained twice daily, ultrasonography was performed once daily (from Leyendecker and Wildt 1983*b*.)

criteria, the following general picture has emerged. During the follicular phase of the menstrual cycle, LH pulses in serum occur at a rate of about one pulse per hour. During the luteal phase, there is a steady decrease of the LH pulse frequency, reaching levels of one pulse every 6–8 hours (Backström *et al.* 1982; Wildt *et al.* 1983, 1984; Reame *et al.* 1984; Veldhuis *et al.* 1984; Crowley *et al.* 1985; Filicori *et al.* 1986). Several authors have described an increase in LH pulse frequency from the early to the late follicular phase of the cycle (Backström *et al.* 1982; Reame *et al.* 1984; Veldhuis *et al.* 1984; Crowley *et al.* 1985; Filicori *et al.* 1986). However, there was no increase in pulse frequency from the mid- to the late follicular phase, the time at which oestradiol concentrations in serum rise dramatically (Backström *et al.* 1982; Wildt *et al.* 1983, 1984; Filicori *et al.* 1986; Steele *et al.* 1986). The difference in pulse frequency between early, mid-, and late follicular phase was absent (Kazer *et al.* 1987) or markedly reduced (Filicori *et al.* 1986) when the nocturnal slowing of the LH pulse frequency during the early follicular phase (see below) was disregarded. No change of LH pulse frequency across the follicular phase was found in the rhesus monkey (Norman *et al.* 1984).

Twenty-four hour sampling regimens have revealed a sleep-associated nocturnal slowing of the LH pulse frequency during the early follicular phase (Kapen *et al.* 1976; Soules *et al.* 1985; Filicori *et al.* 1986; Kazer *et al.* 1987). This could be abolished by opioid antagonism (Rossmanith and Yen, 1987) and disappeared as the follicular phase progressed (Soules *et al.* 1985). There is no information available regarding the possibility of nocturnal slowing of the pulsatile pattern of LH immediately before the onset of the midcycle LH surge when oestradiol levels are maximally elevated (Marshall *et al.* 1988). However, a dramatic nocturnal slowing, with pulses of high amplitude, can be induced in the midfollicular phase by the administration of sufficient oestradiol benzoate to produce late follicular phase levels of the steroid (Marshall *et al.* 1988). Such a slowing of the LH pulse frequency, with large amplitude pulses, at the height of preovulatory oestradiol would be of physiological significance with regard to the frequently observed early morning onset of the LH surge (Edwards *et al.* 1980; Testart *et al.* 1982). Only Marut and co-workers (1981), studying the differences between immuno- and bioassayable LH during midcycle in the rhesus monkey, have observed such an event (Fig. 3 of Marut *et al.* 1981).

During the luteal phase of the cycle, the frequency of the pulsatile pattern of LH secretion is reduced as the luteal phase progresses (Wildt *et al.* 1983; Reame *et al.* 1984; Soules *et al.* 1984; Filicori *et al.* 1986), with the length of the pulse intervals correlating with the duration of the period of serum progesterone elevation rather than the height of the elevation itself (Wildt *et al.* 1983; Crowley *et al.* 1985; Leyendecker *et al.* 1987). That the slowing of the LH pulse frequency is induced by progesterone is

indicated by the production of a luteal-phase pulse pattern when progesterone is administered during the follicular phase of the cycle (Soules *et al.* 1984).

Interdispersed between the pulses of larger size are (1) small elevations of higher frequency and (2) small pulses immediately following a large pulse, creating a 'shoulder'-like appearance of the descending limb of large pulses (Reame *et al.* 1984; Crowley *et al.* 1985). These elevations and shoulders certainly represent additional secretory bursts of pituitary LH and, by inference, of GnRH; however, they are obviously very small and do not, if considered as separate pulses, obliterate the marked difference between the follicular- and luteal-phase pulse patterns.

In addition, the amplitude of the LH pulses varies during the menstrual cycle, declining from the early to the mid- and late follicular phases and reaching high values again during the early and midluteal phases (Wildt *et al.* 1983; Soules *et al.* 1984; Crowley *et al.* 1985). Pulses of FSH in serum are more difficult to detect during the cycle than those of LH. This is probably related to the strong feedback suppression by ovarian steroids and peptides, as well as to the long plasma half-life of this hormone (Yen *et al.* 1972*b*).

As a reflection of the pulsatile gonadotrophin signals to the ovary, the secretion of ovarian steroids is also pulsatile (Backström *et al.* 1982; Djahanbakch *et al.* 1984; Filicori *et al.* 1984; Healy *et al.* 1984; Veldhuis *et al.* 1988). In the periovulatory period, oestradiol fluctuates in a similar manner to LH, although it is not always possible to relate each pulse of oestradiol to a corresponding pulse of LH (Djahanbakch *et al.* 1984). Distinct pulses of progesterone have been observed in the mid- and late luteal phases of the rhesus (Healy *et al.* 1984) and human menstrual cycle (Filicori *et al.* 1984). Longitudinal studies revealed that in the early luteal phase, progesterone secretion is stable and does not show a relationship to episodic LH release. In the mid- and late luteal phases, plasma progesterone concentrations fluctuate rapidly over a 24 h period, changing from very low to very high levels often within minutes. The increments in progesterone closely attend episodes of bioactive LH release (Filicori *et al.* 1984; Veldhuis *et al.* 1988).

IV THE REGULATION OF GONADOTROPHIN SECRETION

The pituitary gonadotrophs are target cells for hypothalamic GnRH, the ovarian steroids, and at least one ovarian peptide (inhibin). In the hypogonadal state, pituitary secretion of LH and FSH is at a constant high level, as reflected in the high serum concentration of both gonadotrophins and a lack of cyclicity. In the eugonadal state, therefore, the cyclic pattern

of gonadotrophin secretion must be related to ovarian hormones acting at the level of the hypothalamic–pituitary unit. While the pituitary is the physiological target organ of hypothalamic GnRH, the interaction of the ovarian steroids with the hypothalamic–pituitary unit is more complex. There is compelling evidence that the ovarian steroids regulate pituitary gonadotrophin secretion by acting predominantly at the level of the pituitary gland. However, by their ability, mediated by the hypothalamic endogenous opioid system, to alter the secretory pattern of GnRH, it is also evident that steroids exert additional, more indirect, regulatory effects on pituitary gonadotrophin release.

1 GnRH

Gonadotrophin-releasing hormone (GnRH), which is derived from a larger precursor molecule (Nikics *et al.* 1985), is secreted from the medio-basal hypothalamus into the portal circulation in an intermittent, pulsatile mode (Clarke and Cummins 1982). The pulsatile secretion is controlled by the arcuate nucleus of the medio-basal hypothalamus, and selective destruction of this region will abolish pituitary gonadotrophin secretion (Knobil 1980*a*). Electrophysiological studies have shown that rhythmic increases in electrical multiunit activity in the region of the arcuate nucleus are coincident with the initiation of LH pulses in serum (Knobil 1980*b*; Wilson *et al.* 1984; Kaufman *et al.* 1985). Since LH pulses in blood correlate with increases of GnRH in portal blood (Clarke and Cummins 1982), there is compelling evidence that increases in hypothalamic electrical multiunit activity represent neuronal activity associated with the release of GnRH. This provides a more direct approach to the study of the regulation of hypothalamic GnRH secretion in the animal model without the necessity of frequent blood sampling (Kaufmann *et al.* 1985; Kesner *et al.* 1987).

At the level of the pituitary, GnRH stimulates synthesis and release of both LH and FSH; the hormones are produced, according to immunohistochemical studies, in the same cell (Childs 1986). GnRH binds to a specific membrane receptor and initiates a subsequent cascade of intracellular events (Wagner *et al.* 1979; Clayton and Catt 1981; Conn *et al.* 1981; Hsueh and Jones 1981; Zolman 1983; Clayton 1984; Conn 1986; Naor and Childs 1986; Naor *et al.* 1989), including the stimulation of messenger RNA for the α- and β-subunits of LH and the β-subunit of FSH (Papavasiliou *et al.* 1986; Zmeili *et al.* 1986; Dalkin *et al.* 1989). GnRH membrane receptor concentration, maximal expression of the LH- and FSH-β-subunit genes, and maximal gonadotrophin release are all determined by both the amount and pattern of pituitary GnRH stimulation, and are modulated by the steroidal environment (Hsueh *et al.* 1979; Lagacé *et al.* 1980; Clayton 1988; Dalkin *et al.* 1989).

In the human and the subhuman primate, the frequency at which the pituitary is stimulated by GnRH under non-luteal conditions is approximately one pulse every hour. The physiological significance of the pulsatile release, and hence of the pulsatile stimulation of the pituitary gonadotroph, was not understood until it was shown that only pulsatile, and not continuous, administration of GnRH to ovariectomized and hypothalamic-lesioned rhesus monkeys could maintain pituitary gonadotrophic function (Belchetz *et al.* 1978). Aberrations from a circhoral pattern of stimulation were found to have profound effects on the relative serum concentrations of LH and FSH. Short intervals between the pulses favoured LH secretion, while longer intervals induced a preferential rise in FSH levels in the hypothalamic-lesioned, ovariectomised rhesus monkey (Wildt *et al.* 1981*a*). These results were confirmed in the orchidectomized rat treated with exogenous testosterone and given GnRH pulses at varying intervals; in addition, the results demonstrated that the differential effects of varying GnRH pulses on LH and FSH secretion were mediated by differential effects on β-LH- and β-FSH-mRNA levels (Dalkin *et al.* 1989). These data indicated a potential physiological role for the modulation of the GnRH pulse frequency in the control of gonadotrophin secretion.

2 Oestradiol

The regulatory role of the ovarian steroids in the secretion of pituitary gonadotrophins has been studied extensively in the human and subhuman primate over the past two decades. It has been shown that the cyclic pattern of the gonadotrophins in serum during the menstrual cycle is largely the consequence of stimulatory and inhibitory effects of ovarian steroids on pituitary gonadotrophin secretion. Thus, oestradiol exerts both inhibitory and stimulatory effects on the secretion of pituitary gonadotrophin, termed negative and positive, (or biphasic), feedback effects. The post-castration rise in LH and FSH serum concentrations results from the withdrawal of the ovarian feedback inhibition (Yen and Tsai 1971*a*; Czygan and Maruhn 1972; Monroe *et al.* 1972*a*, *b*). This feedback can be reinstituted, at least in part, by the exogenous administration of synthetic oestrogen or oestradiol (van de Wiele *et al.* 1970; Wallach *et al.* 1970; Tsai and Yen 1971; Lütjen *et al.* 1986).

The negative feedback of oestradiol on pituitary LH and FSH secretion is very abrupt. Within a few hours after the increase in serum oestradiol, the LH pulse amplitude decreases and LH serum levels start to decline (Yen *et al.* 1972*a*; Knobil 1974; Leyendecker *et al.* 1975*b*). According to recent studies of electrical multiunit activity of the arcuate nucleus in the castrated rhesus monkey (Kesner *et al.* 1987), this abrupt negative feedback and the subsequent rebound in hypogonadal subjects (Yen *et al.* 1972*a*) probably involves the hypothalamic GnRH oscillator in addition to

the pituitary gonadotrophs (Plant *et al.* 1978). If elevated levels of oestradiol are maintained for a prolonged period of time, there is a continuous decline in serum FSH. This contrasts with the serum levels of LH which, after an initial rapid decrease, tend to reach a plateau, or even gradually rise, before the rapid surge (the second component of the biphasic feedback effect) begins. This indicates a differential negative feedback effect of rising oestradiol on pituitary secretion of LH and FSH, with the latter being more sensitive to negative feedback inhibition (Yen and Tsai 1971*b*; Leyendecker *et al.* 1975*b*; Leyendecker 1979; Marshall *et al.* 1983). Interestingly, in a recent study, the cyclic administration of oestradiol valerate and progesterone to hypogonadal patients to produce physiological levels of oestradiol and progesterone in blood, did not result in the full suppression of FSH levels. This was especially evident during the phase of oestradiol substitution alone, indicating the requirement of an additional ovarian FSH inhibitor (Lütjen *et al.* 1986).

In women and all experimental animals studied, the preovulatory LH surge is preceded by a rise in serum oestradiol (Abraham *et al.* 1972; Moghissi *et al.* 1972; Korenman and Sherman 1973; Knobil 1974; Thorneycroft *et al.* 1974; Leyendecker *et al.* 1975*a*; Laborde *et al.* 1976; Pauerstein *et al.* 1978). It has been demonstrated in numerous studies that the administration of oestrogen to castrated or postmenopausal women, and to women in the proliferative phase of the cycle, results in a stimulation of pituitary gonadotrophin release after an initial inhibitory phase. This stimulatory effect has been referred to as the positive feedback of oestradiol. The LH surge provoked by exogenous oestrogens is similar in both shape and duration to the spontaneous midcycle LH peak (van de Wiele *et al.* 1970; Nillius and Wide 1971; Tsai and Yen 1971; Leyendecker *et al.* 1972; Monroe *et al.* 1972*a*, *b*; Yen and Tsai 1972; March *et al.* 1979). Furthermore, neutralization of endogenous oestradiol by the administration of an antiserum directed against oestradiol prevents the induction of the preovulatory LH peak in the rat (Ferin *et al.* 1975). Therefore, there is good experimental evidence that the rising levels of oestradiol secreted from the preovulatory follicle constitute the primary signal for the induction of the preovulatory gonadotrophin rise and subsequent ovulation.

On the basis of the latency of 24–28 h between the administration of oestradiol benzoate and the onset of the LH surge in oestrogen-pretreated, ovariectomized women, and the hormonal pattern of oestradiol and LH in the preovulatory period of the menstrual cycle, a level of 200 pg of oestradiol ml^{-1} of serum has been considered to be the critical threshold for the induction of the preovulatory LH surge in women (Leyendecker *et al.* 1972). In studies of the strength–duration characteristics of exogenously administered oestradiol in the rhesus Macaque, a threshold of around 150 pg ml^{-1}, which had to be sustained for at least 36 h, has been estimated to be necessary for the induction of the LH surge. This acted with a latency

phase of 36–48 h. When supraphysiological levels of oestradiol were produced, the latency phase could be reduced to 24 h (Karsch *et al.* 1973; Knobil 1974). Under physiological conditions, additional factors may be involved in the initiation of the preovulatory LH surge, which may be essentially inhibitory in character. This conclusion is derived from the observations in women with hypothalamic amenorrhoea in whom cycles were induced with high doses of GnRH per pulse: the oestradiol levels surpassed the critical threshold of oestradiol of around 150–200 pg ml^{-1} up to 48 h earlier relative to the onset of the LH surge than was the case in normal cycles or in cycles induced with a lower dose of GnRH (Leyendecker and Wildt 1983*a*, *b*). Clinical observations in the human, as well as experimental studies in the rhesus monkey, also suggest that other controls exist. Some authors have proposed the ovarian secretion of a gonadotrophin surge inhibitor (GnSI) (Schenken and Hodgen 1983, 1986; Sopelak and Hodgen 1984).

The cellular mechanisms involved in the direct effect of oestradiol on pituitary gonadotrophin secretion are not understood completely. *In vivo*, the sensitivity of the pituitary towards identical GnRH stimuli is increased by rising oestradiol concentrations in serum during the normal cycle, or by experimentally raised oestradiol levels maintained over a prolonged period of time (Yen *et al.* 1972*c*; Jaffe and Keye 1974; Keye and Jaffe 1974; Lasley *et al.* 1975; Young and Jaffe 1976). Such effects may be related to the observation that oestradiol regulates positively the GnRH receptor concentration (Clayton 1988). Prior to its facilitory action, however, the rising levels of oestradiol initially inhibit the pituitary response to GnRH (Thompson *et al.* 1973; Keye and Jaffe 1974). This initial inhibitory action of oestradiol may, in the presence of uninhibited synthesis of LH, be the cause of the increased pituitary LH content seen during the late follicular phase of the human menstrual cycle (Bischoff *et al.* 1969) and in experimental studies in the rat and rhesus monkey (Barraclough and Haller 1970; Attardi *et al.* 1980). *In vitro* studies with rat anterior pituitary cells in culture demonstrated both inhibitory (Jutisz *et al.* 1988) and stimulatory actions of oestradiol (Hsueh *et al.* 1979; Lagacé *et al.* 1980) on GnRH-induced LH and FSH release.

3 Progesterone

Progesterone concentrations in serum rise prior to ovulation (Zander 1954) and the ability of progesterone to induce an abrupt release of gonadotrophin from the pituitary has been demonstrated by several investigators using hypogonadal subjects pretreated with synthetic oestrogens (Buchholz *et al.* 1964; Odell and Swerdloff 1968; Nillius and Wide 1971; Leyendecker *et al.* 1972). However, the hypothesis that increasing serum levels of progesterone secreted from the preovulatory follicle is the

primary triggering signal for the midcycle LH release had to be rejected on the basis of studies on the chronological sequence of changes during the periovulatory phase of the human menstrual cycle: the rise in serum progesterone is preceded by, or is concomitant with, the rise in LH (Johansson and Wide 1969; Abraham *et al.* 1972; Leyendecker *et al.* 1972; Thorneycroft *et al.* 1974; Hoff *et al.* 1983). However, on the basis of the short latency (only a few hours) of the progesterone feedback effect, it was suggested that progesterone might play an additional or supporting role in the regulation of the midcycle LH surge (Leyendecker *et al.* 1972). Studies that tried to simulate the endocrine events during midcycle established that progesterone at low levels (levels that are normally surpassed at the initiation of the LH surge) is able to advance the oestradiol-induced LH surge with a latency phase of less than 3 h. Such observations are consistent with the view that rising progesterone from the preovulatory follicle augments or facilitates the primarily oestradiol-induced LH midcycle surge (Leyendecker *et al.* 1972, 1976; Chang and Jaffe 1978; March *et al.* 1979; Helmond *et al.* 1980; Terrasawa *et al.* 1980; Schenken *et al.*, 1982, 1985; Liu and Yen 1983).

The progesterone positive feedback effect only occurs after a well-defined priming by oestradiol. Under experimental conditions, if the rise in progesterone follows that of oestradiol in less than 24 h, the LH surge is blocked (Leyendecker *et al.* 1976). This blocking effect of progesterone was demonstrated under various experimental conditions (Knobil 1974; March *et al.* 1979; Helmond *et al.* 1980). These observations support the view that progesterone, following its facilitory role, exerts a blocking effect on gonadotrophin secretion under physiological conditions (Leyendecker *et al.* 1972). Interestingly, progesterone did not block the oestradiol-inducible LH surge in rhesus monkeys in which gonadotrophin secretion was re-established following hypothalamic lesioning by the pulsatile administration of GnRH (Wildt *et al.* 1981*b*), while it did block the LH surge in control monkeys with an intact nervous system (Pohl *et al.* 1982).

The cellular mechanisms involved in the effects of progesterone on gonadotrophin secretion are not yet defined completely. As in other progesterone target tissues, the priming of the progesterone effects by oestradiol involves the generation of specific progesterone receptors. *In vivo*, progesterone augments the primarily oestradiol-facilitated, GnRH-induced gonadotrophin release (Lasley *et al.* 1975) and the stimulatory effect of progesterone *in vitro* (Lagacé *et al.* 1980) acts with the same latency of less than 3 hours (Leyendecker *et al.* 1976). Progesterone decreases the responsiveness of cultured cells to GnRH (Batra and Miller 1985), an effect which may be related to its ability to regulate negatively the GnRH receptors (Clayton 1988).

4 Inhibin

Inhibin is produced by the granulosa cells of the maturing follicle, by the corpus luteum, and by the placenta (Channing *et al.* 1984; Burger 1988; Tsonis *et al.* 1988). It has been demonstrated that inhibin selectively suppresses pituitary FSH secretion under various experimental conditions (Channing *et al.* 1978; Erickson and Hsueh 1978; Grady *et al.* 1982; Rivier *et al.* 1986). Extracts enriched in inhibin activity, can be used to study the effects of selective suppression of FSH on follicular development and thus, indirectly, the functional role of FSH in the control of the menstrual cycle (Shander *et al.* 1980; Stouffer and Hodgen 1980; Rettori *et al.* 1982; Stillman *et al.* 1983; Channing *et al.* 1984; Schenken *et al.* 1984; Stouffer *et al.* 1984). On the basis of such studies, it was assumed that inhibin would constitute one of the ovarian negative-feedback signals. The characterization of its precise role in the regulation of the menstrual cycle, however, had to await its further identification and isolation (Mason *et al.* 1986; Tsonis and Sharpe 1986; Burger and Igarashi 1988), the development of reliable methods of detection, and a better understanding of the secretory pattern of inhibin in plasma (McLachlan *et al.* 1986, 1987; Robertson *et al.* 1988; Tsonis *et al.* 1988).

Serum concentrations of inhibin are at stable low levels during the follicular phase and rise shortly before the midcycle LH surge. Inhibin levels fall immediately after the LH surge, only to rise again to their maximal height during the luteal phase, paralleling the secretion of progesterone. This suggests that in the human menstrual cycle, ovarian inhibin is predominantly a secretory product of the corpus luteum under the control of LH (McLachlan *et al.* 1989).

5 Other ovarian inhibitors of gonadotrophin secretion

Recently, another protein with FSH-suppressing properties has been isolated from follicular fluid (Robertson *et al.* 1987; Ying *et al.* 1987) and has been termed follistatin. No assays have been developed so far, and the pattern of secretion and the physiology of this ovarian protein remain to be established. The same is true for a putative ovarian gonadotrophin surge-inhibiting factor (GnSIF), which appears to block the oestrogen-inducible LH surge under certain experimental conditions and in treatment regimens of ovarian hyperstimulation (Schenken and Hodgen 1983, 1986; Littman and Hodgen 1984). If this material plays a physiological role by preventing a premature oestradiol-induced LH surge in the normal menstrual cycle, it should be elevated in serum in the late follicular phase and decline immediately prior to the onset of the LH surge. However, this material has not been isolated and identified.

6 The hypothalamic endogenous opioid system

Since hypothalamic GnRH secretion is a prerequisite of normal pituitary gonadotrophic function, any changes in the hypothalamic release of this decapeptide, whether physiological or pathological in character, have profound effects on the pituitary secretion of LH and FSH. For example, chronic elevation of prolactin levels in patients suffering from prolactinomas results in the suppression of gonadotrophin secretion and gonadal function, producing amenorrhoea (Bohnet *et al.* 1976). Evidence that the apulsatility of LH secretion and compromised ovarian function observed in these patients are a consequence of impaired hypothalamic GnRH release comes from the induction of normal gonadotrophin secretion and ovarian function following pulsatile administration of GnRH (Leyendecker *et al.* 1980*a*).

There is considerable evidence that hypothalamic GnRH secretion may be affected by steroids. Volleys of rhythmic hypothalamic electrical multiunit activity, representing hypothalamic GnRH release, were considerably broader in hypogonadal than in intact rhesus monkeys. Administration of oestradiol to castrated animals reduced hypothalamic electrical multiunit activity volleys to the size typical of the eugonadal state. In some instances, the rhythmic appearance of increased electrical activity was blocked abruptly or the frequency was reduced markedly (Kesner *et al.* 1987). In humans with testicular feminization, the chronic administration of oestradiol resulted in a marked reduction of the LH pulse frequency (Veldhuis *et al.* 1985*b*). In addition, exposure of women in the midfollicular phase of the cycle to high levels of oestradiol induced a nocturnal slowing of pulsatile LH release (Marshall *et al.* 1988). Finally, administration of progesterone in the follicular phase induced a luteal LH pulse pattern (Soules *et al.* 1984), while the administration of an anti-oestrogen in the luteal phase increased LH pulse frequency (Maruncic and Casper 1987).

There is compelling evidence that these steroidal influences on GnRH secretion are mediated, at least in part, by the endogenous opiate system. The inhibitive effect of exogenous opiates on gonadal function is well documented. Opiate addiction leads to ovulatory disturbances, including amenorrhoea (Martin *et al.* 1973; Bai *et al.* 1974; Santen *et al.* 1975). Following the demonstration of opiate receptors in the brain and later of specific endogenous ligands, the endogenous opioid system became a focus for research on the central regulation of reproduction. In the rodent (Schulz *et al.* 1981; Forman *et al.* 1983), the subhuman primate (Ferin *et al.* 1982, 1984), and the human (Reid *et al.* 1981*a*), the administration of morphine or synthetic β-endorphin results in a decline in LH levels in the blood, while specific antagonism with β-endorphin antisera or naloxone

results in an elevation of LH. The demonstration of β-endorphin-containing neurons in the medio-basal hypothalamus (Bloch *et al.* 1978; Morell *et al.* 1985) suggested that these effects may be mediated by alterations in the release of hypothalamic GnRH. This view was supported by the finding that perfusion of human fetal hypothalami resulted in an increased secretion of GnRH (Rasmussen *et al.* 1983).

The activity of the hypothalamic endogenous opioid system is controlled by gonadal steroids. The hypothalamic content of β-endorphin and pre-pro-opiomelanocortin messenger RNA is altered by endocrine manipulations such as castration and hormone replacement (Lee *et al.* 1980; Wardlaw *et al.* 1982*a*; Wardlaw 1986; Blum *et al.* 1989; Chowen-Breed *et al.* 1989*a*, *b*). The concentration of β-endorphin in the hypophyseal portal effluent of the rat changes during the oestrous cycle and is reduced following ovariectomy (Sarkar and Yen 1985). In the rhesus monkey, portal blood β-endorphin levels (Wardlaw *et al.* 1980) change during the cycle, with low levels in the follicular and high levels in the luteal phase (Wehrenberg *et al.* 1982). These differences result from the changing sex steroid milieu, as demonstrated by the administration of oestradiol and progesterone to castrated female rhesus monkeys: while portal blood levels of β-endorphin were below the limit of detection, they rose after the administration of oestradiol and reached highest values during the additional administration of progesterone (Wardlaw *et al.* 1982*b*). In the human female, the intravenous administration of β-endorphin resulted in a significant decrease in serum levels of LH in the early follicular phase of the cycle (Reid *et al.* 1983); this response did not occur in postmenopausal women, however, suggesting a reduced endogenous opioid receptor activity in this condition.

An indirect assessment of endogenous opioid activity and its dependence upon the endocrine milieu has been made by the administration of opioid antagonists, such as naloxone and naltrexone, to rodents (Bhanot and Wilkinson 1983), subhuman primates (van Vugt *et al.* 1983, 1984; Ferin *et al.* 1984), and to humans. In general, the administration of opioid antagonists results in an increase of LH secretion both in the luteal phase of a cycle and under conditions of combined oestrogen and progestin treatment, but the antagonists have only a small effect during the follicular phase and no effect in hypogonadal states such as the menopause (Quigley and Yen 1980; Blankstein *et al.* 1981; Moult *et al.* 1981; Reid *et al.* 1983; Snowdon *et al.* 1984; Gilbeau *et al.* 1985; Shoupe *et al.* 1985; Gindoff *et al.* 1988).

A thorough assessment of the endogenous opioid activity across the menstrual cycle was performed by Rossmanith *et al.* (1989) by studying the effects of both 8 and 24 h naloxone infusions during the early and late follicular phases and midluteal phase of the cycle. While 8 h naloxone infusions did not change the LH pulse characteristics in the early follicular

phase, they elevated significantly the pulse frequencies, pulse amplitudes and transverse mean levels in the late follicular phase. In the midluteal phase, the LH pulse amplitude was increased significantly, but pulse frequency and transverse mean levels remain unchanged. While being ineffective in the early follicular phase, the 24 h naloxone infusion elicited a progressive rise in LH pulse amplitude and transverse mean levels in the late follicular phase. The LH pulse frequency remained unchanged in the late follicular phase, while it was increased during the luteal phase. A sustained increase in pulse frequency during the luteal phase has also been observed under prolonged oral administration of naltrexone (Gindoff *et al.* 1988).

Opiate blockade prevents the nocturnal slowing of the LH frequency in the early follicular phase of the cycle (Rossmanith *et al.* 1987), indicating that the sleep-associated effects on gonadotrophin secretion are linked to the endogenous opiate system. In postmenopausal women, opioidergic control of LH secretion can be reinstituted by exogenous oestrogen (Melis *et al.* 1984) and, interestingly, by progestins alone (Casper and Alapin-Rubillowitz 1985). In gonadectomized patients with testicular feminization and under oestrogen replacement for 4–6 weeks, naloxone increased LH pulse frequency from 3 to 5 pulses per 8 h (Veldhuis *et al.* 1985*b*).

In conclusion, these data indicate that the endogenous opioid system participates in the control of gonadotrophin secretion during the menstrual cycle by altering hypothalamic GnRH secretion, and that it is controlled by ovarian steroids. The available data suggests the following general pattern of the endogenous opioid tone and of GnRH secretion across the cycle. In the midfollicular phase, the opioid tone is low and the pulsatile secretion of GnRH secretion is rather uninhibited, occurring at a circhoral frequency. With the progression of the follicular phase and the consequent rise of oestradiol, the endorphin tone is increased; this results in a dampening of the GnRH pulse amplitude and, at the preovulatory height of oestradiol in serum, a possible nocturnal slowing of GnRH pulses (Marshall *et al.* 1988) provoking LH pulses of large amplitude. Elevated progesterone levels in the luteal phase further stimulates the endorphin system, inducing a maximal opioidergic tone at the end of the luteal phase. Accordingly, the frequency of the pulsatile secretion of GnRH and LH declines progressively during the course of the luteal phase. During this time, oestradiol has a permissive effect for the potentiating action of progesterone in increasing the opioid tone (Maruncic and Casper 1987; Nippoldt *et al.* 1989). With the decline in oestradiol and progesterone at the end of the luteal phase, endogenous opioid activity rapidly decreases and a high frequency GnRH pulsatile pattern is resumed within hours (Leyendecker *et al.* 1987). Some residual luteal opioid activity may extend into the follicular phase, however, as indicated by the observation that the

circhoral GnRH/LH pulse frequency does not start immediately, and that there is initially a nocturnal slowing in pulse frequency.

One of the significant characteristics of the endogenous opioid system is that it is activated in a time-dependent, rather than a dose-dependent, manner. While this is obvious during the luteal phase, it is obscured in the follicular phase by the rise of oestradiol which parallels the progression of this phase of the cycle. The involvement of a time component in the oestrogen-induced endogenous opioid tone may be deduced from the observation that, at the end of anovulatory cycles, the pulse frequency decreases in the presence of continuing low oestradiol levels (Clayton *et al.* 1987).

There is considerable physiological individual variability with respect to the level of endogenous opioid activity. Only 50 per cent of women treated with naloxone infusions during the preovulatory phase of the cycle responded with a premature dramatic rise in LH levels, resulting in ovulation (Rossmanith *et al.* 1988). The non-responders, presumably with no significant endogenous opioid activity during this phase of their cycle, were characterized in their control cycles by higher preovulatory oestradiol levels than the responding women.

V ENDOGENOUS GnRH DEFICIENCY

A complete absence or severe reduction of pulsatile gonadotrophin release in women results in the impairment of follicular maturation, anovulation, and amenorrhoea (Yen *et al.* 1973; Leyendecker 1979; Leyendecker and Wildt 1983*a*; Wildt *et al.* 1983). While this occurs physiologically before puberty or during pregnancy and lactation, it is pathological in other periods of reproductive life. Since there is substantial indirect evidence that the cause of this kind of amenorrhoea is a reduced stimulation of the anterior pituitary gland by GnRH, and since GnRH is secreted from the hypothalamus, it is referred to as hypothalamic amenorrhoea (Yen *et al.* 1973; Leyendecker 1979; Leyendecker *et al.* 1980*a*). The term hypothalamic amenorrhoea was coined by Klinefelter *et al.* (1943) to describe amenorrhoea of suprapituitary origin. Due to some cases described in the original publication, however, it was confined later to psychogenic amenorrhoea (Reifenstein 1946). Today, it is ascribed mostly to patients suffering from secondary amenorrhoea. The term hypogonadotrophic hypogonadism, though less specific, is often used to describe cases of primary hypothalamic amenorrhoea. In this review, the term hypothalamic amenorrhoea is used in its original, broader sense and consequently applies to patients with lesions of the pituitary stalk or hypothalamus, or with anorexia nervosa or Kallmann's syndrome, as well as to idiopathic and stress-related 'functional' amenorrhoea. Future insights into the

underlying aetiologies will certainly enable us to develop a more specific nomenclature.

Since endogenous GnRH cannot be measured reliably in peripheral blood, direct evaluation of hypothalamic function is not possible at present. Therefore, the diagnosis of hypothalamic amenorrhoea is based essentially on the exclusion of other causes of amenorrhoea, such as hyperprolactinaemia, hyperandrogenaemia, primary ovarian failure, and genital tract defects, as well as internal and neurological diseases. Primary pituitary failure, a rare incidence, is excluded by the ability to stimulate gonadotrophic function by pulsatile administration of GnRH (Leyendecker and Wildt 1981). Recently, systematic studies have been performed to document the reduced hypothalamic GnRH activity in patients with hypothalamic amenorrhoea, by measuring LH pulsatility in blood (Wildt *et al.* 1983; Crowley *et al.* 1985; Reame *et al.* 1985; Veldhuis *et al.* 1985*a*; Khoury *et al.* 1987). The LH pulse pattern of these patients was very heterogeneous and ranged from apulsatility, over prepuberal and luteal phase-like patterns, to normal follicular phase patterns. Moreover, individual patients exhibited varying patterns when studied over a period of several months (Crowley *et al.* 1985).

Wildt and co-workers (1983) studied 20 patients suffering from hypothalamic amenorrhoea and correlated the severity of hypothalamic amenorrhoea as assessed by established functional tests (Table 3.1, Fig. 3.2) (Leyendecker *et al.* 1979; Leyendecker *et al.* 1983*a*) to the individual pulse pattern, the overall LH and FSH levels during a 24 h period, as well as to the results of ovarian ultrasonography (Fig. 3.3). The number of LH pulses was lowest in patients with grade 3c hypothalamic amenorrhoea but increased gradually until a value comparable to that of normal menstrual cycle was reached in grade 2 patients. Only in patients with grade 3b amenorrhoea (i.e. showing a prepuberal response to iv GnRH) did an increase in pulse frequency during sleep become apparent; in all other patients, the pulses were distributed evenly between asleep and awake periods. The amplitude of some LH pulses, however, was considerably larger during sleep than during the awake periods in subjects with grades 3b, 3a, and 2 amenorrhoea. Overall LH and FSH levels increased in parallel with the number of LH pulses up to grades 3a and 2, but failed to reach values typical for the early follicular phase of the cycle. In patients responding to clomiphene, the levels of LH and particularly FSH declined again; this may be attributed to feedback inhibition by the elevated levels of oestradiol found in these patients.

Follicular development up to the large antral stage was identified by ultrasound (Fig. 3.3). Follicular size increased in parallel with the number of LH pulses. The number of follicles ranged from very low or undetectable in grade 3c patients, to numbers comparable to those of the early follicular phase of normal menstrual cycles in clomiphene-positive (grade

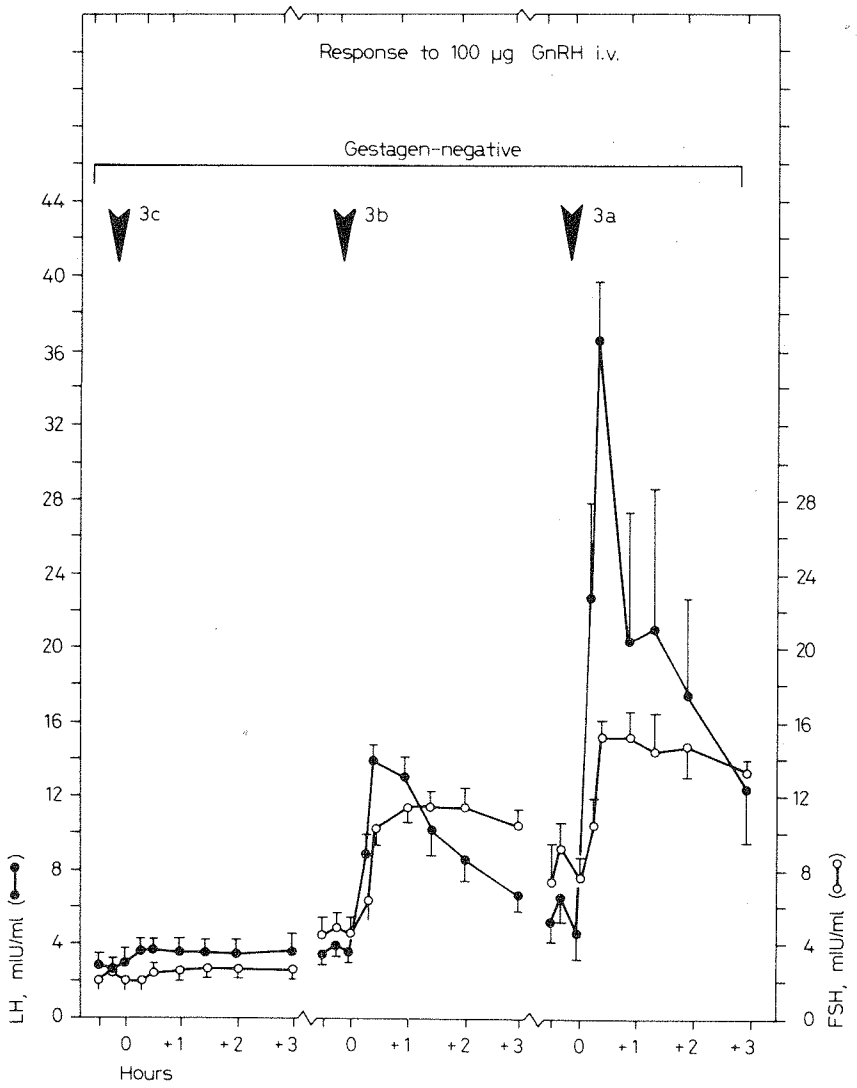

Fig. 3.2 The LH and FSH response to an IV bolus of 100 µg GnRH in women with hypothalamic amenorrhoea grades 3a, 3b, and 3c (progestagen negative) (from Leyendecker and Wildt 1983*a*).

1) patients. Thus, hypothalamic amenorrhoea is characterized by a reduced frequency and amplitude of gonadotrophin secretion and is reflected by the concomitant reduction of ovarian follicular growth. A reduction in frequency of pulsatile gonadotrophin, and by inference GnRH secretion, is characteristic of the most severe grades of hypothalamic

Table 3.1 *Grading of hypothalamic amenorrhoea on the basis of clomiphene*, gestagen†, and GnRH‡ tests, respectively.*

Grade	Result of test
1	Clomiphene positive (bleeding)
2	Gestagen positive (bleeding) Clomiphene negative (no bleeding)
3	Gestagen negative (no bleeding), but with pituitary response to 100 μg of GnRH iv:
3a	'adult' response
3b	'prepuberal' response
3c	no response

* The clomiphene test involves the administration of 100 mg of clomiphene for 5 days and is assessed by occurrence of a bleeding in due time. The bleeding might be preceded by a clomiphene-induced ovulatory cycle, a cycle with luteal insufficiency, or an anovulatory cycle.
† The gestagen test involves the adminstration of a daily oral dose of 10 mg medroxyprogesterone-acetate for 10 days and is assessed by the occurrence of a withdrawal bleeding.
‡ The GnRH test consists in the iv administration of 100 μg of GnRH and is assessed by the measurement of LH and FSH serum levels (Fig. 3.2).

amenorrhoea, while a reduction in amplitude characterizes less pronounced grades. Santen *et al.* (1978) proposed that increased sensitivity to negative feedback was the mechanism responsible for eugonadal secondary amenorrhoea. It is conceivable that, in the presence of a reduced hypothalamic drive, the pituitary is more sensitive to negative feedback than is the case under normal hypothalamic activity. Additionally, endogenous opioid inhibition may be increased in these patients and may be activated further during and following an oestrogen challenge, resulting in a prolonged suppression of gonadotrophin levels.

The close correlation between the functional grading system, based on the clomiphene, gestagen, and GnRH tests, and the endocrine and sonographic findings in patients with hypothalamic amenorrhoea, indicates the suitability of these tests for the assessment of residual hypothalamic function in clinical practice and experimental research. In 58 women with secondary hypothalamic amenorrhoea, the tests revealed a wide distribution of grades, ranging from grade 1 to 3b. Grade 3c was found only once in secondary hypothalamic amenorrhoea. On the other hand, in 17 women with primary hypothalamic amenorrhoea, mainly grades 3b and 3c were observed. Only one patient with primary hypothalamic amenorrhoea presented with grade 3a (Leyendecker and Wildt 1983*a*).

While the difference in severity of hypothalamic impairment between secondary and primary hypothalamic amenorrhoea points to the probability of different underlying aetiologies, the individual pulse pattern of LH

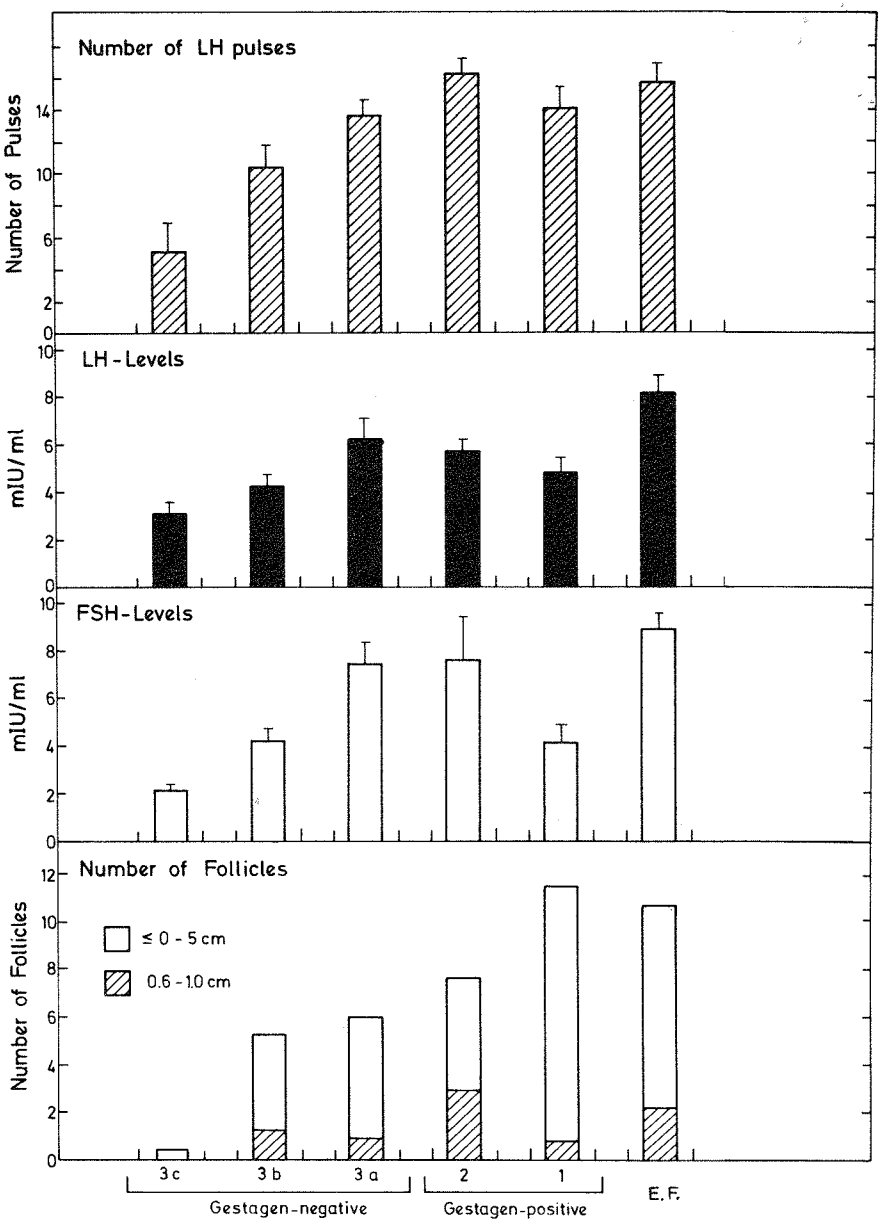

Fig. 3.3 Number of LH pulses in 24 h, mean LH and FSH levels over the 24 h sampling period and number of class I and class II follicles in patients suffering from hypothalamic amenorrhoea (graded according to their response to GnRH, gestagen, and clomiphene administration). Bars indicate mean ± SEM. The corresponding values for the early follicular phase (E.F.) (day 3–7) of 13 normal menstrual cycles are given for comparison and represent values of 8 h sampling periods. The number of follicles given for E.F. represents the maximum number observed for each class of follicles. (From Wildt *et al.* 1983.)

is not a useful indicator in this respect. Individuals with secondary hypothalamic amenorrhoea may exhibit different pulse patterns over the course of time (Crowley *et al.* 1985) and, moreover, patients with similar pulse patterns may respond differently to the administration of an opioid antagonist (Khoury *et al.* 1987).

Although women with secondary amenorrhoea are more likely to respond to the administration of naloxone with an increase in LH pulse frequency and LH levels than patients with primary hypothalamic amenorrhoea (unpublished observation), and may even respond with the development of ovulatory cycles under the chronic oral administration of naltrexone (Wildt and Leyendecker 1987), about 50 per cent of women with secondary hypothalamic amenorrhoea did not respond to the administration of naloxone. The absence of responsiveness in these women was not related to a previous history of either weight loss or to strenuous exercise (Quigley *et al.* 1980*b*; Khoury *et al.* 1987), conditions known to be unrelated to increased endogenous opioid activity (Grossmann *et al.* 1982; Dixon *et al.* 1984; Giusti *et al.* 1988). While the non-responders in the study of Quigley *et al.* (1980*b*) had lower oestradiol levels than the responders, such an association could not be assessed in the study of Khoury *et al.* (1987).

In conclusion, a variety of conditions, such as physical and psychogenic stress, weight loss, and anorexia nervosa, as well as genetic and idiopathic factors and trauma, may result in an impairment of ovarian function. While a variety of different pathophysiological mechanisms may be involved, the common denominator in all cases is a reduced hypothalamic secretion of GnRH, the degree of which can be precisely and usefully graded on the basis of functional tests.

VI PULSATILE ADMINISTRATION OF GnRH

The elucidation of the physiological significance of the pulsatile stimulation of the pituitary gland by GnRH (Belchetz *et al.* 1978), the demonstration of the pituitary as the primary site of gonadal feedback (Nakai *et al.* 1978), and the subsequent concept of the solely permissive, although obligatory, role of hypothalamic GnRH in the regulation of the primate menstrual cycle (Knobil 1980*a*; Knobil *et al.* 1980), together opened the perspective of a new therapeutic approach to the treatment of infertility in GnRH-deficient states that previously had been treated with gonadotrophins in a rather unphysiological way (Leyendecker 1979; Leyendecker *et al.* 1980*a*, *b*; Leyendecker and Wildt 1983*a*). Shortly after its introduction, this new mode of treatment proved to be effective in inducing ovulation and subsequent pregnancy in patients with hypothalamic amenorrhoea (Leyendecker *et al.* 1980*b*; Shoemaker *et al.* 1981; Berg *et al.* 1983; Hurley *et*

al. 1984; Loucopoulos *et al.* 1984; Mason *et al.* 1984; Weinstein *et al.* 1984; Jansen *et al.* 1987*a*, *b*; Gompel and Mauvais-Jarvis 1988). At the same time, the pulsatile administration of GnRH served as a new tool for the study of the central control of the menstrual cycle in the primate (Knobil 1980*a*; Wildt *et al.* 1981*a*, *b*, *c*; Pohl *et al.* 1982; Norman *et al.* 1983; Plant 1986) and the human (Crowley and McArthur 1980; Leyendecker *et al.* 1980*a*; Leyendecker and Wildt 1981; Valk *et al.* 1981; Santoro *et al.* 1986*a*, *b*; Soules *et al.* 1986).

In the subhuman primate, by hypothalamic lesioning (Nakai *et al.* 1978), by pituitary stalk transection (Ferin *et al.* 1979) and by placement of silastic sheets between the cut ends of the stalk (Knobil 1980*a*), experimental conditions can be created that allow the study of the effects of administered GnRH on the pituitary–ovarian axis without the interference of endogenous GnRH. In contrast, hypothalamic amenorrhoea in women constitutes a pathophysiological continuum of different grades of severity with different grades of residual hypothalamic GnRH activity (Leyendecker and Wildt 1983*a*). Thus, for physiological studies on the central control of gonadotrophin secretion in women, only the most serious grades (such as grades 3c and 3b), or cases of pituitary stalk transection, can serve as useful experimental models. In such apulsatile or nearly apulsatile patients, ovulatory cycles were induced by the pulsatile administration of GnRH at an unvaried frequency of one pulse every 60–90 min and at unvaried doses as low as 1.25 μg to 1.5 μg pulse^{-1} (Leyendecker *et al.* 1980*a*, *b*; Crowley and McArthur 1980; Leyendecker and Wildt 1981; Reid *et al.* 1981*b*; Skarin *et al.* 1982; Leyendecker and Wildt 1983*a*; Santoro *et al.* 1986*a*) (Figs 3.4 and 3.5). These GnRH-induced cycles did not differ from normal menstrual cycles in duration, in the cyclic pattern of pituitary gonadotrophins and ovarian steroids in blood, or in their potential to result in pregnancy (Fig. 3.6) (Leyendecker and Wildt 1986; Gompel *et al.* 1988; Wilcox *et al.* 1988). This supports the concept, developed initially in the subhuman primate (Knobil *et al.* 1980) and subsequently extended to the human (Leyendecker *et al.* 1980*a*), of a solely permissive role for hypothalamic GnRH in the regulation of gonadotrophin secretion.

Systematic studies using different dose levels of GnRH per pulse (2.5–5.0 μg pulse^{-1} versus 15–20 μg pulse^{-1}, Leyendecker and Wildt 1983*a*, *b*; and 25 ng kg^{-1} body weight versus 100 ng kg^{-1} body weight, Santoro *et al.* 1986*a*) demonstrated a dose-response relationship between the dose per pulse administered and the pituitary–ovarian response. With a dose of 1 μg pulse^{-1} infused intravenously over 1 min every 90 min, no follicular maturation could be induced. The permissive threshold started around 1.25 μg pulse^{-1} (Leyendecker and Wildt 1983*a*). In the study of Santoro *et al.* (1986*a*), a dose per pulse of 25 ng kg^{-1} body weight resulted in follicular and luteal phase steroid levels that were below those of normal control

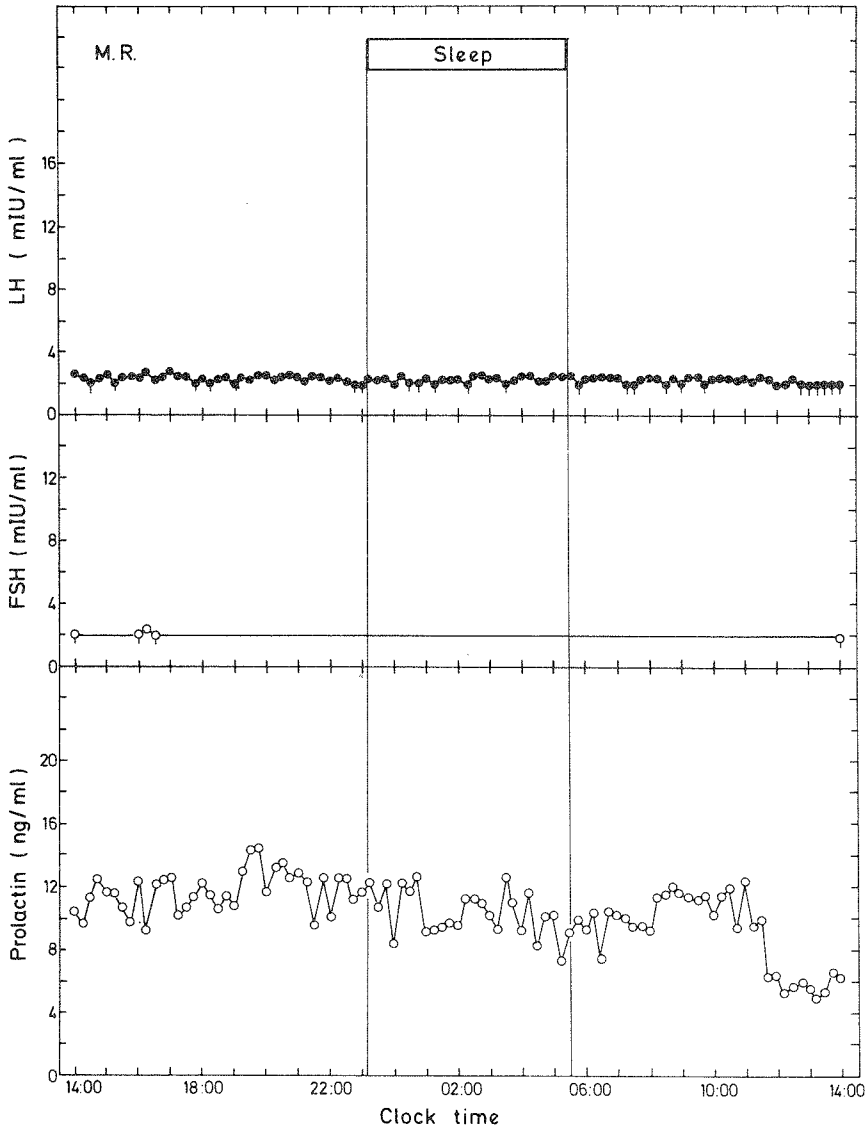

Fig. 3.4 The 24 h secretory pattern of a patient suffering from primary gestagen-negative hypothalamic amenorrhoea grade 3c. The pituitary stalk had been transected and removed with parts of the hypothalamus during surgery for removal of a craniopharyngeoma. Note the complete absence of LH pulses and the consistently elevated levels of prolactin. Vertical lines underneath data points indicate levels below assay sensitivity. (From Wildt *et al.* 1983.)

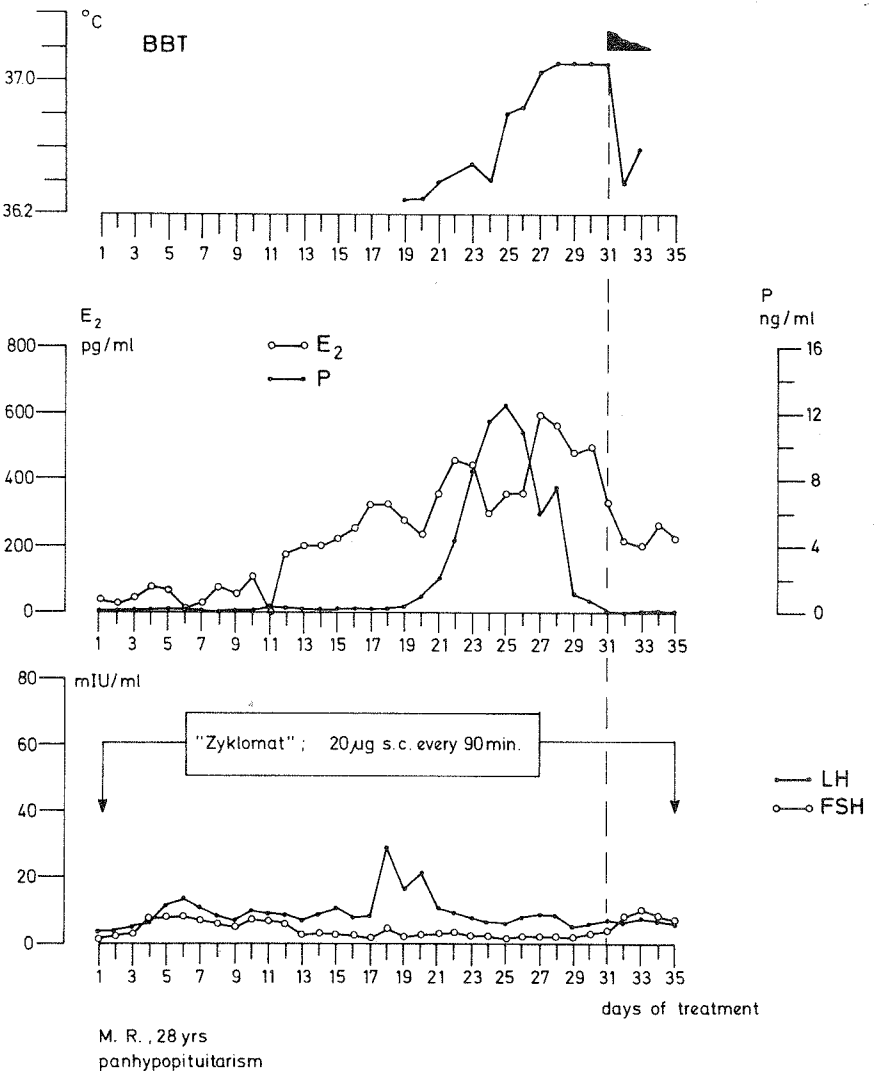

Fig. 3.5 Basal body temperature and the serum levels of oestradiol, progesterone, LH, and FSH during a cycle induced by the sc administration of 20 μg of GnRH every 90 min in patient M.R. suffering from panhypopituitarism following removal of a craniopharyngeoma. This experimental cycle demonstrates that, by prolonged pulsatile administration of GnRH, primary pituitary failure could by excluded. Furthermore, it demonstrates that the concept of the permissive function of hypothalamic GnRH in the regulation of the menstrual cycle, developed in the rhesus monkey, can be extended to the human. (From Leyendecker and Wildt 1981.)

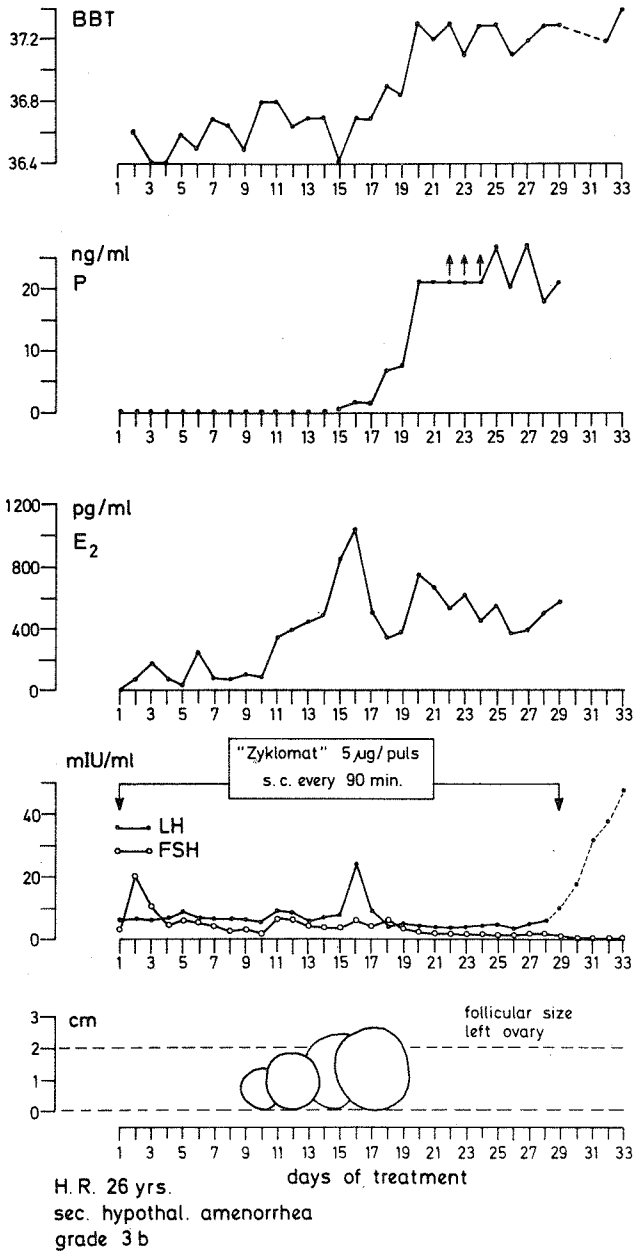

Fig. 3.6 Induction of ovulation and pregnancy in a patient suffering from secondary hypothalamic amenorrhoea grade 3b using pulsatile GnRH at a dose of 5 μg pulse^{-1} subcutaneously. The basal body temperature, the size of the dominant follicle, progesterone, oestradiol, LH, and FSH levels are depicted. The rise in LH after day 27 represents cross-reaction with human chorionic gonadotrophin hCG (unpublished).

cycles, while a dose per pulse of 100 ng kg^{-1} body weight induced steroid levels slightly above normal. Marshall and Kelch (1986) suggested that the common use of supraphysiological doses of GnRH in treatment regimens may have obscured a physiological need for increased GnRH activity in the follicular phase of the cycle. However, the occurrence of ovulation in cycles induced with hourly, low-dose, GnRH pulses (25 ng kg^{-1} body weight, Santoro *et al.* 1986*a*) suggests that there is no need for any increased GnRH activity at midcycle: instead, it appears that a low dose of GnRH, that is not sufficient to produce normal follicular maturation (as indicated by the subnormal steroid levels), may still induce the midcycle LH surge.

The studies of pulse administration of GnRH to women with hypothalamic amenorrhoea has also raised questions concerning the relationship between the threshold requirements for oestradiol and the initiation of the preovulatory LH surge. According to initial concepts discussed earlier (p. 103), the LH midcycle peak is induced, after a latency phase of 36–48 h, when the oestradiol levels in serum are maintained above a threshold of about 150 pg ml^{-1} for a duration of at least 36 h. In the rhesus monkey, supraphysiological levels of oestradiol shorten this latency phase considerably (Knobil 1974) and in the human, the LH surge may be advanced by administration of oestrogen in the late follicular phase (Dhont *et al.* 1974). However, the data obtained from women treated with supraphysiological doses of GnRH suggest that the control of the LH surge may be rather more complicated. Using GnRH doses of 15–20 μg $pulse^{-1}$, preovulatory peak levels of oestradiol of around 1000 pg ml^{-1} were obtained, with the threshold levels of 150–200 pg ml^{-1} being surpassed more than 72–96 h prior to the onset of the LH surge (Leyendecker and Wildt 1983*a*) (Fig. 3.7). These observations suggest that either the oestradiol positive feedback threshold is variable or the execution of the positive feedback is delayed by an as yet unknown component. This additional control factor must be inhibitory in nature and may be an ovarian LH-surge-inhibiting substance (Schenken and Hodgen 1986). Alternatively, it may be an inherent component in the dynamics of the GnRH and oestradiol interaction at the pituitary level, such that the strength–duration characteristics of the oestradiol positive-feedback effect on LH secretion may also depend upon the strength of the hypothalamic GnRH drive, as suggested earlier (Leyendecker and Wildt 1983*b*).

An increased hypothalamic drive, whether exogenous or endogenous in nature, may increase the chance of polyovulation and of heterozygous multiple pregnancies. Thus, Martin and co-workers (1984) observed that women who gave birth to dizygous twins had higher levels of LH and FSH and oestradiol in the early and midfollicular phases of their cycles (suggesting an increased hypothalamic drive) than did controls. Similarly, unphysiological stimulation of the pituitary by the administration of a dose

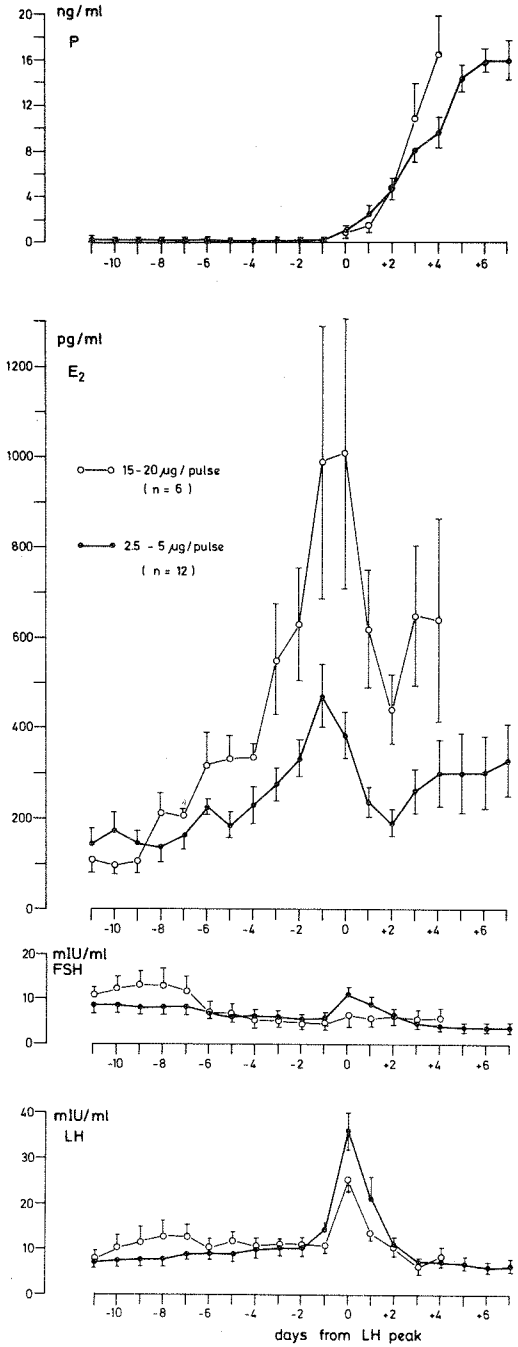

Fig. 3.7 Serum concentration of FSH, LH, oestradiol, and progesterone in patients with hypothalamic amenorrhoea grade 3b, comparing results when GnRH doses of 15–20 μg pulse^{-1} or 2.5–5.0 μg pulse^{-1} were applied (from Leyendecker and Wildt 1983*a*).

of more than 5 μg of GnRH per pulse may result in superovulation and multiple pregnancy (Leyendecker and Wildt 1983*a*). Most of the reported cases of overstimulation and multiple pregnancy following GnRH, however, are related not to unphysiological doses of GnRH, but rather to the induction of ovulation in patients with hypothalamic amenorrhoea of low grade (e.g. grades 3a and 2) (Bogchelman *et al.* 1982; Heinemann *et al.* 1984). In our study of pulsatile GnRH administration to patients with hypothalamic amenorrhoea, four of five multiple pregnancies occurred in patients with low-grade secondary hypothalamic amenorrhoea treated at doses of 2.5–5 μg pulse^{-1}. No multiple pregnancies occurred in patients with primary, and generally severe-grade (e.g. 3b and 3c), hypothalamic amenorrhoea at doses up to 20 μg pulse^{-1} (Leyendecker and Wildt 1986). In these patients with low-grade hypothalamic amenorrhoea and considerable endogenous GnRH activity (Yen *et al.* 1973), the initiation of pulsatile administration of GnRH induces a series of unphysiologically large gonadotrophin pulses. The results would be consistent with the intraovarian mechanisms of follicle selection and suppression (which normally result in a single ovulation) being able to tolerate relatively high levels of FSH in the early follicular phase, provided that the actual rate of increase in FSH levels is relatively slow. Therefore, the rate of FSH increase would be an important determinant of mono- or polyovulation.

As discussed above, the daily pattern of serum levels of LH, FSH, oestradiol, and progesterone in the luteal phase of cycles induced with an unvaried pulsatile administration of GnRH does not differ from that of normal cycles. While there is clear experimental evidence that the corpus luteum requires appropriate gonadotrophic stimulation (van de Wiele *et al.* 1970; Leyendecker and Wildt 1981; Groff *et al.* 1984; Mais *et al.* 1986; Zelesnik and Hutchison 1986) (Fig. 3.8), the functional role of the reduction of GnRH/LH pulse frequency during the normal luteal phase is less clear. To investigate this, a follicular phase pulsatile pattern of LH secretion was induced during the luteal phase of normal cycles by the circhoral administration of GnRH (Soules *et al.* 1987) and by the oral administration of naltrexone (Gindoff *et al.* 1988). None of the functional characteristics of the luteal phase, such as its duration and steroid levels, were changed and there was no influence on the functional characteristics of the subsequent cycle. This is also in keeping with the observation that prolonged stimulation with unvaried pulsatile GnRH results in repetitive normal cycles in the primate (Knobil 1980*a*) and in the human (Skarin *et al.* 1982). The potential role of GnRH pulsatility in limiting luteal function is included in the following section.

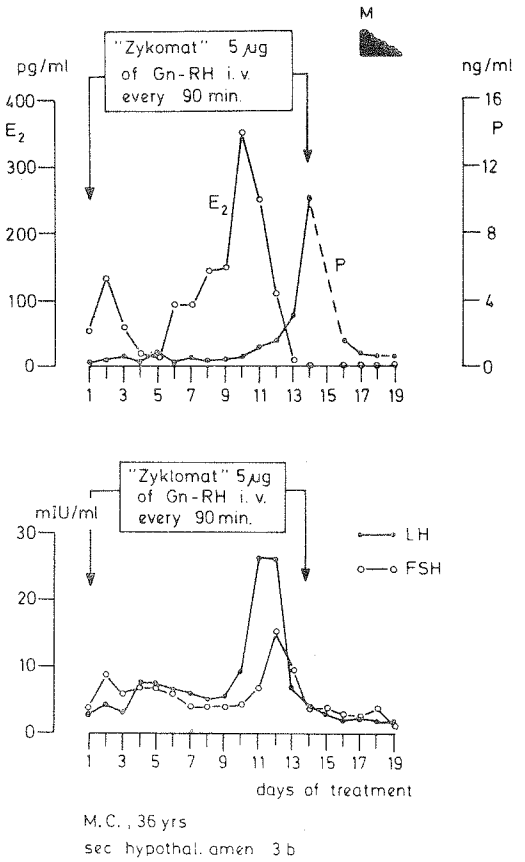

Fig. 3.8 The pattern of LH, FSH, oestradiol and progesterone in a patient suffering from secondary hypothalamic amenorrhoea grade 3b. Pulsatile administration of GnRH was terminated on day 14 of treatment. (From Leyendecker and Wildt 1981.)

VII A MODEL OF THE CENTRAL CONTROL OF THE REGULATION OF FOLLICULAR MATURATION AND OVULATION IN THE HUMAN

The analysis of the frequency of LH pulses during the mid-, late, and periovulatory phases of the menstrual cycle, and the demonstration that normal cycles can be induced in endogenous-GnRH-deficient subjects by the administration of unvaried pulses of GnRH at a circhoral frequency, strongly suggest that the function of the hypothalamic GnRH in regulating the menstrual cycle is permissive in character, with the negative and

positive feedback effects of ovarian hormones being exerted mainly at the level of the pituitary gland.

With the demise of the corpus luteum at the end of an infertile cycle, oestradiol, progesterone, and inhibin concentrations in serum fall to early follicular phase levels. The hypothalamus and the pituitary gonadotrophs are both liberated from ovarian feedback restraints, allowing the hypothalamus to re-initiate the circhoral pulsatile secretion of GnRH. This, in turn, results in a gradually increased secretion of FSH, and then of LH, from the formerly maximally inhibited pituitary gonadotrophs. The slow increase in FSH and LH serum levels stimulates a cohort of follicles and ensures that the intraovarian mechanisms of selection of the dominant follicle and the suppression of all other recruited follicles are not overridden by too high a tide of gonadotrophic stimulation. Immediately following selection of the dominant follicle, a dichotomy in the synthesis and secretion of LH and FSH is enforced upon the pituitary gonadotrophs by the secretory products of the growing follicle. While the dominant follicle evolves from being dependent on both FSH and LH to being dependent predominantly on LH, the FSH levels are suppressed progressively by rising oestradiol and finally also by inhibin, ensuring that no additional LH-stimulatable follicles exist at midcycle.

At the level of the pituitary gland, the rising levels of oestradiol modulate the pituitary effects of GnRH on LH synthesis and release. The effect of oestradiol is such that, after surpassing a certain threshold, the synthesis and release of LH are dramatically activated, resulting in the LH midcycle peak accompanied by a small peak of FSH. The initiation of the gonadotrophin midcycle peaks may be controlled additionally by a preovulatory decrease of an ovarian gonadotrophin surge-inhibiting factor. Rising LH levels induce the luteinization of the preovulatory follicle and a small increase of serum progesterone, which in turn augments the effects of oestradiol on the midcycle LH release. Following an intermediate fall or step-like arrest in their concentrations during the ovulatory period, oestradiol, inhibin, and progesterone levels rise again after the formation of the corpus luteum, to reach maximal levels during the midluteal phase. This is followed by gradual decline to low levels again at the end of the luteal phase. During the periovulatory and the luteal phases, the preovulatory follicle/corpus luteum becomes completely LH/hCG dependent and completely FSH independent. It is unaffected, therefore, by the maximal suppression of FSH serum levels during the late follicular and the luteal phases.

The effect of inhibin during the luteal phase is to suppress FSH levels maximally and thus to prevent the presence of any additional ovarian structure that might be susceptible to LH and hCG stimulation. The administration of human postmenopausal gonadotrophins in the luteal phase and in early and late pregnancy has shown that the ovary certainly

has the potential to respond with follicular development at these times (White and Bradbury 1965; diZerega and Hodgen 1979; Zelesnik and Resko 1980; Serafini *et al.* 1985). The potential hazard of additional follicles responding to the stimulatory action of LH/hCG is often experienced in hMG/hCG-induced ovulation and pregnancy. Another possible role of inhibin extends into the cycle following an infertile one: the maximal suppression of FSH in the luteal phase may prevent the formation of any surge of FSH at the initiation of the new cycle, which might override the intraovarian regulation of mono-ovulation.

Since a normal cycle, involving all these stimulatory and inhibitory feedback effects of ovarian secretory products on gonadotrophin secretion, can be obtained by the unvaried pulsatile administration of GnRH in GnRH-deficient subhuman primates and women, what is the functional role of the endogenous opioid system in modulating GnRH/LH pulse frequency and amplitude? The principle role of this system appears to be to temporarily and reversibly reduce hypothalamic GnRH activity, and thereby to reduce ovarian activity, at selected times. This occurs at the end of an infertile cycle, during pregnancy and lactation, and during conditions that lead to stress, and subsequent functional, amenorrhoea. Specific endocrine stimuli for the activation of the endogenous opioid system are elevated oestradiol, elevated progesterone following oestradiol priming (Mais *et al.* 1987; Maruncic and Casper 1987; Nippoldt *et al.* 1989), and chronically elevated prolactin levels (Quigley *et al.* 1980*a*). Corticotropin-releasing factor (CRF) may also act in this respect (Gindoff and Ferin 1987). However, this view does not exclude other mechanisms of reversible suppression of central GnRH activity that do not involve the endogenous opioid system.

While the significance of reduced reproductive potential is obvious during pregnancy and lactation, and is conceivable during stress, the significance of the activation of the endogenous opioid system with a reduction of the hypothalamic GnRH secretion during the cycle is not so readily apparent. The involvement of the opioid system is more obvious when considering the final stages of sexual maturation, when opioidergic control of gonadotrophin secretion is significant (Mauras *et al.* 1986). The following sequence of events can be suggested.

From the time around menarche to the stage of full sexual maturation, the opioidergic inhibitory tone steadily declines to a level compatible with normal ovulatory function. The hypothalamic–pituitary–ovarian axis passes through stages of 'hidden anovulatory cycles' prior to menarche (Leyendecker and Wildt 1983*a*), anovulatory cycles and menstrual cycles with corpus luteum insufficiency, until normal ovulatory function is established. A 'hidden anovulatory cycle' prior to menarche results when, in the presence of an elevated endogenous opioid tone, the increased secretion of oestradiol from a stimulated follicle further increases the

central opioidergic activity, which in turn suppresses hypothalamic GnRH secretion and withdraws gonadotrophin stimulation from the follicle. An anovulatory cycle is initiated when, in the presence of a gradually reduced endogenous opioid tone, the endometrial threshold is surpassed by rising oestradiol from the maturing follicle. Towards the end of the cycle, the elevated oestradiol levels, by increasing the endogenous opioid tone, may induce a slow-frequency GnRH/LH secretory pattern (Clayton *et al.* 1987) which terminates follicular stimulation. Atresia and menstruation ensue, ending the cycle. Cycles with corpus luteum insufficiency may result when the opioid-induced slow-frequency GnRH pulses produce high-amplitude LH pulses, which induce some luteinization. The subsequent slight increase in serum progesterone initiates an LH surge, which in turn induces ovulation, but produces an insufficient corpus luteum. This would explain the occurrence of midcycle LH peaks in cycles with luteal insufficiency but in which the preovulatory oestradiol levels never reached the normal positive-feedback threshold (Leyendecker *et al.* 1975*a*). In the situation close to full maturation, the central opioidergic tone is decreased further and the largely unrestrained hypothalamic GnRH secretion allows sufficient LH and FSH secretion to produce normal follicular maturation. Increasing preovulatory oestradiol, however, activates the endogenous opioid system, which imposes a nocturnal slowing on the pulsatile GnRH release; this results in high-amplitude LH pulses, especially at the end of the slowing phase (Marshall *et al.* 1988). The high-amplitude LH pulses induce a slight rise of progesterone which facilitates the preovulatory LH surge. The surge is therefore initiated in the early morning (Edwards *et al.* 1980; Seibel *et al.* 1982; Testart *et al.* 1982). This involvement of progesterone in the initiation of the LH surge represents a fail-safe mechanism for the production of the surge (Odell and Swerdloff 1968). It was probably in patients exhibiting such a degree of endogenous opioid tone, that the infusion of naloxone was able to induce LH and progesterone increments and a subsequent LH surge and ovulation (Rossmanith *et al.* 1988).

At full maturation, the endogenous opioid tone is low during the follicular phase and is not measurably activated at the end of the follicular phase, as indicated by the lack of response of LH levels to naloxone infusions (Rossmanith *et al.* 1988). The LH surge is induced by oestradiol and the subsequent rise of progesterone augments this (Leyendecker *et al.* 1972; Leyendecker *et al.* 1976). During the luteal phase, the elevated progesterone dramatically increases the endogenous opioid tone and progressively reduces the GnRH/LH pulse frequency, thereby helping to terminate luteal function as early as possible in an infertile cycle (Maruncic and Casper 1987). This potential physiological role of the endogenous opioid system in terminating an infertile cycle has been obscured partly by the fact that the duration of the luteal phase of cycles induced by unvaried pulsatile GnRH is not significantly longer than in normal cycles, and partly

by the notion that the corpus luteum has an inherent physiological life of about 14 days. The physiological life of the corpus luteum, however, really extends far into midpregnancy, although its function is only required until the completion of the luteo-placental shift. Any termination of luteal function before completion of the luteol-placental shift can be regarded, therefore, as premature, resulting from an imbalance between the gonadotrophic stimulation available and the increasing minimal threshold requirements of the corpus luteum. Such a 'supply and demand' mechanism of gonadotrophic stimulation of the corpus luteum would control the further progress of reproduction: insufficient gonadotrophin stimulation would be indicative of either a failure of conception or impaired function of the conceptus and would lead to luteolysis and menstruation. The sensitivity of this control mechanism may be of particular importance in relation to the high incidence of early pregnancy loss in humans (Wilcox *et al.* 1988). The process of premature luteal breakdown is really initiated in the second half of the luteal phase when the gonadotrophic support to the corpus luteum decreases. Thus, the pulsatile pattern of GnRH secretion and pituitary LH release during the luteal phase of the cycle may be seen as having evolved as providing the minimal gonadotrophic stimulation required to maintain the corpus luteum until the provision of additional support by trophoblastic chorionic gonadotrophin.

VIII CONCLUSIONS

At the beginning of a new cycle in women, the hypothalamic oscillator is nearly completely liberated from opioid restraints. During the follicular and periovulatory phase, hypothalamic GnRH is secreted at a permissive level that allows, without changes of GnRH pulse frequency or amplitude, full follicular maturation, ovulation, and corpus luteum formation. Ovarian negative and positive feedbacks occur almost entirely at the level of the pituitary gland. Negative feedback is exerted by the ovarian steroids and inhibin, with FSH being more sensitive than LH to the feedback inhibition. This results in the dichotomy of pituitary gonadotrophin secretion which starts in the early follicular phase and which is of utmost importance with respect to restricting the growth of additional follicles. With the progressive dichotomy of gonadotrophin secretion, the dominant follicle changes from a structure dependent on both FSH and LH into a completely LH/hCG-dependent structure. The positive-feedback effect on LH, which is responsible for ovulation and corpus luteum formation and which is induced by oestradiol and augmented by progesterone, occurs at the level of the pituitary gonadotrophs and does not require an increase in hypothalamic GnRH activity.

During a nearly mature or mature menstrual cycle, the opioid system,

by its ability to slow down GnRH pulse frequency and thus to generate high-amplitude LH pulses, may control the onset of the progesterone positive-feedback fail-safe mechanism and thereby control the timing of the onset of the LH surge in the early morning. The progesterone fail-safe feedback is likely to be activated towards the end of opioid-dependent and sleep-associated slowing of the frequency of LH pulses, leading to ovulation about 36 h later. It may be a remnant of a linkage between human reproduction and circadian rhythms.

Opioid modulation of GnRH pulse frequency may also be important for the generation of a preferential rise in FSH which stimulates the recruitment of a new cohort of follicles after an infertile cycle or following suppression of gonadal function, such as in pregnancy or lactation. A selective stimulation of FSH may also be an important component of the earlier stages of female sexual maturation. During the mature menstrual cycle, however, the mechanism promoting preferential FSH secretion is overtaken by selective feedback inhibition of FSH.

Another physiological role of the opioid modulation of GnRH secretion may be to terminate luteal function in infertile cycles, although the significance of this effect may be partly obscured by a 'supply and demand' component in luteolysis. In addition, the endogenous opioid system has a physiological role in the temporary, reversible reduction of reproductive potential during pregnancy, lactation, and adverse conditions such as chronic stress. Some reproductive disorders, such as hyperprolactinaemia, may take advantage of these physiological control systems.

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