OPIOID PEPTIDES IN THE FEMALE REPRODUCTIVE SYSTEM: PHYSIOLOGICAL IMPLICATIONS

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SUMMARY

• All endogenous opioid peptides derive from three precursor molecules i.e. proopiomelanocortin, proenkephalin and prodynorphin. The endogenous opioid peptides exert their biological effects through opioid receptors. Each endogenous opioid peptide exhibits higher binding affinity towards a specific type of opioid receptors. Current evidence suggests that endogenous opioid peptides play important regulatory roles in reproduction. Endogenous opioid peptides are present through the hypothalamic-pituitary-gonadal axis. The hypothalamic opioidergic mechanism represents one of the important central control systems of gonadotropin-releasing hormone and gonadotropin release. Opioids mediate the sex steroid effect exerted on gonadotropin-releasing hormone and luteinizing hormone secretion and play a crucial role in the integration of several neuroendocrine mechanisms. There is also evidence that suprahypothalamic mechanism enhances endogenous opioid inhibition of gonadotropin-releasing hormone. The genes of the endogenous opioid peptides are also expressed in peripheral reproductive tissues such as the endometrium and placenta. At least part of the endogenous opioid peptides effects may be paracrine or autocrine in nature. The possible roles of opioids in various physiological processes of the female reproductive system are also reviewed.

• Opiate effects on human reproductive function were recognized long before the isolation of endogenous peptides with opioid activity (1). Since this discovery, research has expanded greatly and a vast body of literature now documents the physiology and pathology of endogenous opioid modulation of hormonal and other aspects of reproduction. Endogenous opioid peptides (EOF) are present throughout the hypothalamic-pituitary-gonadal axis as well as in the endometrium and placenta. Current evidence suggests that EOF play important regulatory roles in reproduction. At least part of EOF effects may be local, paracrine or autocrine in nature. It is thus possible that local opioids may be part of microregulatory loops within each reproductive organ. This review will concentrate on the human data but will refer to animal studies if important differences occur or if human data are unavailable.

OPIOID PEPTIDES AND PRECURSORS

• Met-enkephalin and leu-enkephalin were the original opioids isolated from porcine brain (1). They share the opioid-active N-terminal sequence Tyr-Gly-Gly-Phe and differ only in their C-active residue methionine and leucine, respectively. All opioid peptides share the sequence of either met- or leu-
enkephalin at their N-terminal, but possess C-terminal extensions of variable length, which may confer greater potency (2), increased stability against degradation (3) and different receptor specificity. Analysis of DNA sequence in recent years has allowed the prediction of the amino-acid sequence of three precursor molecules (4).

Proopiomelanocortin (POMC) is a glycoprotein of 31 kD and is the precursor for adrenocorticotropic hormone (ACTH), α-melanocyte-stimulating hormone (α-MSH), ß-lipotropin and also ß-endorphin but does not appear to be further processed to met-enkephalin. The precursor product relationship was first established by radiolabeling studies on AtT-20 cells which derive from an ACTH-producing tumor or mouse pituitary. The final posttranslational products of POMC precursor molecule differ between tissues expressing POMC gene. Thus, in anterior pituitary, the tissue with the highest concentration of POMC mRNA and POMC derived peptides, ACTH is the most predominant posttranslational product of POMC and plays an essential role in the hypothalamic-pituitary-adrenal axis.

Proenkephalin is the major biological source of met-enkephalin and its extended forms (met-enkephalin-Arg-Phe, met-enkephalin-Arg-Gly-Leu and peptide E) and one source of leu-enkephalin. Proenkephalin-derived peptides, in contrast to POMC, are all opioids in nature. Each molecule of pro-enkephalin contains seven opioid peptides with the met- or leu-enkephalin active core.

Prodynorphin (proenkephalin B) contains 3 copies of leu-enkephalin and its extended forms dynorphin, rimorphin (dynorphin B), leumorphin and α-neo-endorphin. Dynorphin A, a seventeen amino-acid peptide, was the first posttranslational product of prodynorphin to be isolated (2,5). Dynorphin A and its two smaller fragments have the highest potency of all known endogenous opioid peptides in the guinea pig ileum bioassay, a characteristic attributed to their high-affinity for the K-opioid receptor. In fact it appears that they are the only endogenous K-receptor agonists known (6).

The three opioid precursors are of similar molecular size and also contain a number of sequence homologies, suggesting that all may have originally derived from an ancestor opioid gene (Table 1).

### Table 1. Precursor molecules of opioid peptides

<table>
<thead>
<tr>
<th>Precursors</th>
<th>Opioid peptides</th>
</tr>
</thead>
<tbody>
<tr>
<td>POMC (Proopiomelanocortin)</td>
<td>ACTH</td>
</tr>
<tr>
<td>Proenkephalin A</td>
<td>met-enkephalin</td>
</tr>
<tr>
<td>Proenkephalin B (Prodynorphin)</td>
<td>dynorphin A</td>
</tr>
</tbody>
</table>

### Table 2. Opioid receptors

<table>
<thead>
<tr>
<th>Receptor type</th>
<th>Agonist</th>
<th>Antagonist</th>
</tr>
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<tbody>
<tr>
<td>mu (µ)</td>
<td>morphine</td>
<td>naloxone</td>
</tr>
<tr>
<td></td>
<td>ß-endorphin</td>
<td></td>
</tr>
<tr>
<td>kappa (κ)</td>
<td>ketocyclazocine</td>
<td>naloxone</td>
</tr>
<tr>
<td></td>
<td>dynorphins</td>
<td>nor-binalorphinine</td>
</tr>
<tr>
<td></td>
<td>ß-endorphin</td>
<td></td>
</tr>
<tr>
<td>delta (δ)</td>
<td>enkephalins</td>
<td>naltrindole</td>
</tr>
<tr>
<td>epsilon (ε)</td>
<td>ß-endorphin</td>
<td>naloxone</td>
</tr>
<tr>
<td>sigma (σ)</td>
<td>N-allylnormetazocine</td>
<td></td>
</tr>
</tbody>
</table>

OPIOID RECEPTORS

- The EOF exert their biological effects through opioid receptors. Each EOF exhibits higher binding affinity towards a specific type of opioid receptor. A variety of types of opioid receptors have been described. Behavioral studies in dogs suggested that opioids could be divided into (J-, K- or o- agonists (7). Studies with opioid peptides using bioactivity in peripheral tissue preparations (8), or binding studies of radioactively labelled ligands in brain tissue (9) confirmed the existence of (a, 8, K and possibly ε- receptors (Table 2).

The δ-receptor, so named because of its activation by morphine, mediates analgesia, bradycardia and hypothermia. The K-receptor activated by ketocyclazocine, appears to be responsible for sedation and depression of flexor reflexes, but has no involvement in skin twitch reflex or pulse rate. A third class of receptors was named o for its activation by SKF 10,047 (N-allylnormetazocine). For an effect to be considered consequent to opioid receptor activation, the effect must be reversed by
the classical opioid antagonist naloxone or its more potent analogue, naltrexone. The \( \mu \)- and K-receptor-mediated events are readily reversible by naltrexone (7), while physiological events affected by \( \delta \)-receptors do not (10). An additional class of opioid receptor identified (11) is activated by the enkephalins and is named \( \kappa \).

Other receptors considered to be opioid on the basis of their sensitivity to naloxone have been identified in binding studies, but a clear functional significance associated with them is lacking. Among these receptors are \( \delta \) and \( \omega \) receptors mediating analgesia and \( \mu \) receptors mediating respiratory depression (12). These assumptions are based upon the observation that naloxone antagonizes analgesia but not the respiratory depression induced by morphine. Unfortunately, there is to date no antagonist that can reverse the respiratory depression without affecting the analgesia.

Subtypes of \( \kappa \)-receptors have also been proposed (13). There may be as many as four subtypes of the \( \kappa \)-receptor based upon evidence from radioligand binding studies (14). Opioid receptors termed \( \epsilon \) were originally identified as those mediating inhibition of electrically evoked twitch in rat vas deferens (15). These receptors were believed to be selectively activated by \( \beta \)-endorphin. Finally, \( \chi \) receptors first described in binding studies in rat brain (16) were defined as having high-affinity for naloxone, but low affinity for another opioid antagonist, diprenorphine. A functional role for this class of opioid receptors has not yet been proposed.

**LOCALIZATION OF OPIOID PEPTIDES AND RECEPTORS**

- Opioids are localized throughout the central nervous system in specific neuron tracts, and separated on the basis of their known precursors (17). All opioids are concentrated in the hypothalamus, pituitary, periaqueductal grey matter and spinal cord. Hypothalamic (\( \beta \)-endorphinergic neurons originate largely in the arcuate nucleus from where they project to the median eminence as well as other parts of the brain. Enkephalinergic neurons exist in several hypothalamic nuclei while prodynorphin-derived peptides are mostly concentrated in the suprachiasmatic and paraventricular nuclei, although some are also present in the arcuate nucleus and posterior hypothalamus (18). \( \mu \) - and K-receptors are present in hypothalamus in roughly equal numbers but \( \delta \)-receptors account for less than 10% of opioid binding (9).

The pituitary contains large amounts of \( \beta \)-endorphin in the anterior pituitary corticotroph cells and prodynorphin-derived opioids in the posterior pituitary vasopressin neurons (18). Met-enkephalin is present in both anterior pituitary somatotrophs (19) and posterior pituitary oxytocin neurons (20).

Outside the central nervous system opioids are concentrated in the adrenal medulla and other parts of the sympathetic nervous system, and in neurons in the gut. Smaller quantities are also described in the testis and pancreas (21). \( \beta \)-endorphin and met-enkephalin both circulate in human plasma (22).

The EOF genes are expressed in most tissues involved in mammalian reproduction. POMC gene is expressed in the hypothalamus (23), gonads (24), endometrium (25) and placenta (26). In all these tissues P-endorphin appears to represent the main posttranslational product of POMC. POMC mRNA and P-endorphin are present in the granulosa, luteal and intestinal cells of female gonads, in the endometrial cells of uterus, and in the placental syncytiotrophoblast. The proenkephalin gene is expressed in hypothalamus and gonads, and the prodynorphin gene in hypothalamus, anterior pituitary gonadotroph cells, male and female gonads, placenta, and endometrium.

**OPIOID PEPTIDES AND CENTRAL REGULATION OF THE FEMALE REPRODUCTIVE SYSTEM**

- **Prolactin**

Administration of morphine (27), the enkephalin analogue DAMME (FK 33-824) (28), and P-endorphin (29) all cause prompt release of prolactin in man. In the rat, P-endorphin is a more potent secretagogue than met-enkephalin (30), or dynorphin (31), and intraventricular administration of antisera for P-endorphin lowers both basal and stress-induced prolactin secretion (32), suggesting that P-endorphin is indeed the opioid involved. This is compatible with the naloxone sensitivity of opioid stimulation in man (28) which suggests involvement of \( \mu \)- or \( \delta \)-selective opioids. The opioid effect is blocked by the administration of dopamine agonists and potentiated by dopamine antagonists (33), suggesting that opioids act by inhibition of dopamine release, the major prolactin inhibitory factor from the median eminence. This concept is supported by direct experimental evidence in the rat, where opioids decrease hypothalamic dopamine turnover (34) and release (35). Most studies have failed to find any direct effect of opioid peptides on prolactin secretion by isolated pituitary cells in vitro. In the rat, naloxone lowers basal, stress-induced and suckling-induced prolactin secretion, suggesting an important physiological role for opioids on prolactin secretion (36). In contrast, most studies have failed to demonstrate an effect of naloxone, even at high dosage, on basal or stress-induced release of prolactin in normal subjects (37), nor an elevated prolactin level in the puerperium (37), or in patients with prolactinomas (38). A minority of studies has however reported an inhibition of basal or stress-related prolactin release (39). High doses of naloxone may abolish the exercise-induced release of prolactin in highly trained male athletes (40). Naloxone infusion (1.6 mg per h) may stimulate pulsa-
tile prolactin release in women during the late follicular and mid-luteal phases of the menstrual cycle (41) and in women under oral contraception (42), but not in early follicular or late luteal phase, or in hypogonadal women. This pulsatile release of prolactin is synchronous with that of LH, suggesting that a common mechanism, presumably involving luteinizing hormone-releasing hormone (LHRH), mediates the release of both LH and prolactin under these circumstances. Indeed, LHRH may stimulate prolactin release, at least in postmenopausal women (43), and in vitro evidence suggests that this might involve paracrine gonadotroph-lactotroph interactions (44). In spite of the existence of stimulatory and possibly inhibitory opioidergic mechanisms for control of prolactin secretion, no clear physiological or pathological role has emerged.

Gonadotropins

The predominant opioid modulation of gonadotropin secretion is inhibitory. Chronic opiate addiction has been long recognized to cause amenorrhoea and infertility in the female (45). Morphine and other opiates decrease serum LH (27). Inhibition of follicle-stimulating hormone (FSH) is less consistently noted, perhaps due to its longer half life in the circulation. Naloxone acutely increases serum LH and possibly FSH in both sexes, suggesting tonic inhibitory opioid control of gonadotropin secretion (46). Studies on LH pulsatility indicate an increase in both frequency and amplitude of LH pulses with naloxone in both sexes (46). In females, this effect is most marked in the late follicular and particularly mid-luteal phase of the menstrual cycle, and opioids may thus mediate the slowing of LH pulsatility seen at this time. In normally menstruating women, the opioid activity of LH secretion shows a fluctuation dependent on the menstrual cycle phase. In the early follicular phase, the opioid activity is low, increasing proportionately with the ovulatory peak of LH, then remaining high during luteal phase (47). The high progesterone levels during luteal phase of menstrual cycle are suggested to increase the tone on LH pulsatility (48). Low doses of naloxone are required to modulate LH release, suggesting primary involvement of \( \delta \)- or \( \epsilon \)-receptors (48). However, in the immature rat, hypothalamic injections of antisera to both (3-endorphin and dynorphin) raise LH (49), suggesting that more than one opioidergic pathway might be involved. Opioid effects occur at hypothalamic level and involve modulation of LHRH release. Thus opioids have no effect on the LH response to the LHRH test (50). LHRH antagonists block naloxone stimulation in the rat (51), naloxone stimulates the LHRH release from human hypothalamus in vitro (52), and morphine decreases hypothalamic LHRH content (53).

In man, as in the rat, opioidergic regulation mechanisms appear to be closely connected to the feedback of gonadal steroids and LHRH secretion. In postmenopausal women, the endogenous opioid tone is impaired, as reflected by the lack of naloxone effect on plasma LH levels (54). This neuroendocrine response is absent in women with physiological and surgical menopause (54). Estrogen/progesterin therapy affects the normal opioidergic tone and restores a concomitant LH release after naloxone injection (54). This observation in women has been supported by studies in animals. In fact, naloxone abolishes the inhibitory effect of sex steroids on LH secretion in rats (55). This effect disappears after gonadectomy, and naloxone is no longer able to stimulate LH secretion in male and female rats. These data suggest that opioidergic regulation of LH secretion is abolished by the removal of sex steroids (56). On the other hand, women under hormonal contraception are also characterized by the absence of the LH response to naloxone (57). Gonadal steroid feedback on LHRH may thus be mediated via opioid pathways; however, evidence as to whether opioids can inhibit LH secretion in postmenopausal woman is contradictory. It is therefore possible that the presence of gonadal steroids might be necessary for the activation of a separate opioid pathway which inhibits LH release.

Changes in hypothalamic opioid activity have been implicated in a number of pathological conditions under which gonadotropin secretion is reduced. Patients with oligomenorrhea or amenorrhea secondary to hyperprolactinemia have normal mean gonadotropin levels but only infrequent LH pulses of large amplitude (58). Infusion of naloxone in such patients promptly restores normal LH pulsatility (59) without altering the serum prolactin. This suggests that prolactin inhibits LH secretion via opioidergic mechanisms. Similar LH responses to naloxone have been reported in patients with so-called hypothalamic amenorrhea and in the amenorrheic athletes (60). The correlation is further confirmed by data showing that induction of ovulation in amenorrheic patients restores a normal response of LH to naloxone (61). Disturbances of menstrual cycle are also correlated with changes in endogenous opioid activity. The treatment with naltrexone is effective in some patients with hypothalamic amenorrhea in restoring the ovulatory menstrual cycle (62). Weight loss-related amenorrhea is also associated with disturbances in LH pulsatility, irreversibly by naloxone administration (63). In contrast to the rat, the majority of patients with anorexia nervosa show no gonadotropin response to naloxone (63), and those patients who do respond may have another preexisting cause for amenorrhea (64). Similarly, patients with Kallman’s syndrome and idiopathic hypopituitarism show no response to naloxone (65). In polycystic ovary syndrome, the absence of response of LH to naloxone has been reported. In these women the hyperandrogenic state leads to an amenorrheic state which, in some patients, is opioid-dependent (66). In comparison, menstruating hyperandrogenic patients have a normal response to naloxone during luteal phase of menstrual cycle, even in the presence of
Opioid peptides in female reproductive system

of the primary defect of hypothalamic GnRH production (63).

• Central opioid-sex steroid interactions

A close relationship between the central endogenous opioid system and sex steroids is confirmed by several studies conducted both in vivo and in vitro. Using autoradiography and immunocytochemistry, it has been found that arcuate nucleus of the hypothalamus contains estradiol-concentrating neurons as well as p-endorphin-like immunoreactivity. A subpopulation of the arcuate nucleus P-endorphin neurons is receptive to the estradiol (77). Centrally microinfused P-endorphin modifies mammalian "sexual behaviour" and abolishes the estrogen-dependent lordosis in castrated and estrogen primed rats (78). It has also been reported that estrogen reduces hypothalamic POMC mRNA (79). A small population of hypothalamic P-endorphin producing neurons contains progesterone. This finding may indicate that the stimulatory effect of progesterone on GnRH and LH secretion is mediated at least in part by the endogenous opioid system. The reduction of opioid binding sites in mediobasal hypothalamus and preoptic area is linked to progesterone-induced LH surge (80). An increase of hypothalamic P-endorphin content has also been observed following a chronic treatment with various progestins in ovariecetomized rats (81).

Taken together these studies indicate that the endogenous opioid system is involved at least in part in the central control of GnRH and gonadotropin release. Opioids mediate the sex steroid effect exerted on GnRH and LH secretion and play a crucial role in several neuroendocrine mechanisms involved in reproduction.

• Ovaries

Immunoreactive P-endorphin is present in the ovaries of many species including rodent (82) and human (83). Most of this material has the molecular weight of authentic P-endorphin while a significant percentage exhibits higher molecular weight (84). It has been reported that the prodynorphin mRNA is present in rat ovaries and that its concentration changes markedly during the estrus cycle (85). Immunocytochemical and in situ hybridization data show that the granulosa and luteal cells appear to be the predominant source of ovarian POMC-derived opioids. The evaluation of P-endorphin in follicular fluid shows a preovulatory increase of p-endorphin levels, despite constant ACTH and p-lipotropin concentrations throughout the different phases of menstrual cycle (87). This pattern appears again in supero-
vulated follicles, supposing the existence of a relationship between follicle maturation and POMC expression. Ovarian P-endorphin (88) and POMC mRNA rise sharply on the evening of proestrus, suggesting that its synthesis is regulated by gonadotropins and gonadal steroids (89) (Table 3).

The physiological significance of opioids in growing follicle and in corpus luteum is still unknown (82). Recent data indicate that the immature follicle contains lower P-endorphin amounts than the preovulatory, fully mature follicle exposed to gonadotropin stimulation. This confirms that P-endorphin is the end product of POMC in ovulatory dominant follicle. The increase of follicular P-endorphin in preovulatory follicle could be related to the isolated (3-endorphin peak described in peripheral circulation around the ovulatory period (90). The absence of a concomitant (3-lipotropin rise has been interpreted as a phenomenon independent of the anterior pituitary secretion (90). The finding that plasma P-endorphin peak occurs only in the ovulatory cycles (87) led to the hypothesis that "ovarian endorphin" could contribute to peripheral P-endorphin secretion (90). The finding that plasma P-endorphin peak occurs only in the ovulatory cycles (87) led to the hypothesis that "ovarian endorphin" could contribute to peripheral P-endorphin levels in certain circumstances, such as the ovulation.

A pattern opposite to that described for p-endorphin exists as far as ACTH and its products are concerned. This indicates that not only the synthesis of POMC mRNA (82) but also the posttranslational processing of POMC are a function of the ovarian cycle. In this respect, the changes of y-endorphin in follicular fluid are worth mentioning. Since y-endorphin levels are higher in immature follicles than in superovulated ones and luteinized unruptured follicles, it could be hypothesized that maturational processes of granulosa cells inhibit the conversion of P-endorphin into Y-endorphin. The peptide pattern in luteinized unruptured follicles is superimposable to that of superovulated follicles with the only exception of P-endorphin levels which remain lower. Since the increased concentration of P-endorphin is a typical sign of follicle maturation, it is possible to speculate that this opioid is involved in the ovulation process. Different substances, including progesterone and prostaglandins, regulate locally the follicle rupture through a coordinate action on both plasmin production and muscularis mucosae contraction (91). Follicular P-endorphin could therefore interfere with this mechanism in view of the possible presence of opiate receptors in ovarian cells (92).

In conclusion, all these data demonstrate that the POMC processing in the follicle changes as a function of ovarian endocrine activity. Moreover, the reduced P-endorphin levels in the luteinized unruptured follicles support the hypothesis that these peptides could be involved in mechanisms leading to follicle collapse (83).

### Uterus

All three opioid peptide precursors are synthesized in mammalian uterus. Current experimental evidence suggests that the POMC gene is expressed in human endometrium and the size of its transcript is similar to that present in pituitary (25). The prodynorphin gene is also expressed in human endometrial cells, giving a transcript similar in size to that present in the rat anterior pituitary (93). In the rat uterus, the prodynorphin gene is also expressed, but the size of its transcript (2.2 Kb) is 200 bases smaller to that reported in rat hypothalamus (86). The proenkephalin mRNA has also been detected in the macaque uterus with higher levels observed during the proliferative phase of the menstrual cycle (94), suggesting a regulatory role of estrogens. On the other hand, in the rodent uterus, progesterone has been shown to increase both met-enkephalin secretion and proenkephalin A mRNA levels, while the higher levels of proenkephalin expression occur during metestrus and diestrus of normally cycling rats (95). These data propose highly divergent regulatory mechanisms for proenkephalin expression between primate and rodent uteri. It appears also that progesterone stimulates endometrial P-endorphin since the latter is only detectable in the proliferative human endometrium (96) and increases the secretion of P-endorphin in the uterus of ovariectomized gilts (97). In Ishikawa human endometrial cells, which has been shown to be a good in vitro model for the study of the effects of steroid hormones on human epithelial endometrium, the apparent molecular weight of the secreted P-endorphin is that of authentic p-endorphin (25), while the bulk of secreted dynorphin had an apparent molecular weight of 8 kD (93). Estrogen and glucocorticoids decrease P-endorphin secretion from Ishikawa cells (25), while both progesterone and dihydrotestosterone do not significantly affect it (25). The antiprogestin-antiglucocorticoid RU-486 acts as an agonist, diminishing P-endorphin secretion possibly via glucocorticoid receptors (25). On the other hand, the secretion of endometrial dynorphins is not affected by any of these steroids, while LHRH induces a significant increase of dynorphin secretion without affecting that of P-endorphin (93). These data suggest that the regulation of endometrial opioids

<table>
<thead>
<tr>
<th>Organs</th>
<th>POMC</th>
<th>PDYN</th>
<th>PENK</th>
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<tbody>
<tr>
<td>Ovaries</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Uterus</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Placenta</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Abbreviations: POMC - proopiomelanocortin, PDYN - prodynorphin, PENK - proenkephalin.
is type-specific. Thus it is possible that each type of endometrial opioid participates in different local homeostatic loops and exerts distinct paracrine effects.

The role that the endometrial opioids plays within the uterine cavity is still largely unknown. In general, it is postulated that opioids of peripheral tissue have local, paracrine or autocrine effects. Endometrial p-endorphin may also act locally, since it has been shown that K-opioid receptors are present in human endometrial cells (98) and p-endorphin increases the concentration of estrogen receptors in uterine epithelial cells, thus affecting the sensitivity of endometrium to estrogens (99). In addition, endometrial opioids may exert a number of other functions, such as relaxation of smooth muscle (100). Smooth muscle bundles contained in the supportive ligaments as well as in the circular muscle system of the uterus undergo contractions during the preovulatory peak of estrogens. This coordination of muscular contractions plays an important role in properly orienting the ovary with the infundibulum of the fallopian tube at the time of ovulation, and any pathological condition interfering with this coordination may be a cause of dysmenorrhea. The preovulatory peak of estrogens by decreasing endometrial p-endorphin release could potentially facilitate the uterine contractions necessary for efficient movement of the ovum into the tube or the transport of the sperm through the uterine cavity.

Numerous findings propose an immunological role of opioid peptides (101). Recent data show that the corticotropin-releasing hormone (CRH) gene is expressed in normal and tumoral epithelial cells of human endometrium (26). The coexpression in endometrial epithelial cells of both CRH and POMC suggests that endometrial CRH may have an autocrine or paracrine effect on endometrial POMC-derived peptide secretion, as in placenta and testes (26). The involvement of CRH in the inflammatory process has been recently established. CRH has been detected in inflammatory sites, whereas immunoneutralization of CRH attenuates the inflammatory response (102). It is possible that endometrial CRH participates in the regulation of immunological events taking place within the uterine cavity, specifically in egg nidation and implantation. It is known that (i) the human blastocyst secretes prostaglandin E, (PGE,) (103), (ii) PGE, promotes the attachment and implantation of the blastocyst (103), (iii) PGE, is a major inducer of CRH expression in the placenta (104), and (iv) POMC-derived P-endorphin possesses immunosuppressive properties (101). From these data the following scenario during egg implantation could take place: the blastocyst secretes PGE, at the site of nidation, which among other things, stimulates the production of CRH from the endometrium; subsequently, CRH participates in local events culminating in the attachment of the egg; at the same time, endometrial CRH may also suppress inflammatory response by augmenting the production of local, endometrial P-endorphin, which will produce a confined immunosuppression at the site of nidation, inhibiting the rejection of the semixenograft. Thus, another potential site of action of endometrial P-endorphin could be its participation, along with glucocorticoids, interleukins (26) and CRH, in endometrial immunological events associated with egg implantation. Here, it is worth mentioning that oc-MSH exerts an antiinflammatory effect (105) and GnRH and LH negatively influence B lymphopoiesis (106).

- **Placenta**

The human placenta is rich in a variety of opioids including P-endorphin (102), met- and leu-enkephalin and dynorphin (103) (see Table 3). Synthesis of P-endorphin, p-lipotropin and ACTH, as well as a number of higher molecular weight precursors has been demonstrated by pulse-chase experiments in cultured placental trophoblasts (104). The posttranslational processing of placental POMC is similar to that in hypothalamus and gonadal tissues since most of the POMC-derived peptides have the physicochemical characteristics of α-MSH and P-endorphin (103). However, as with other reproductive tissues, placental POMC mRNA is by about 300 nucleotides shorter than the putative counterpart (107). Prodynorphin-derived opioids have been also detected in human placenta (103). Immunohistochemically, placental POMC-derived peptides are localized in the villous syncytiotrophoblast, where most of placental pituitarylike hormones are synthesized (108). It has been found that POMC-derived peptides are being secreted from perfused human placental slices in vitro and from placental trophoblasts in culture (104), and the secretion rate of P-endorphin is higher than that of ACTH (26).

A number of substances affect the secretion of placental POMC-derived peptides. CRH-binding sites are present in human placenta (109) and CRH stimulates the secretion of all placental POMC-derived peptides (26). Glucocorticoids suppress de novo synthesis of POMC in anterior pituitary corticotrophs (110), but they appear not to affect the secretion of POMC-derived peptides from perfused placental slices in vitro. The oxytocin gene is expressed in reproductive tract tissues and an oxytocin-like substance has been identified in human placenta. The secretion of all placental POMC-derived peptides is stimulated by oxytocin in a dose-dependent manner (26). Prostaglandins increase plasma and amniotic fluid levels of p-endorphin in pregnant women undergoing prostaglandin-induced therapeutic abortion at the second trimester of pregnancy (111). In addition, prostaglandins stimulate the secretion of placental POMC-derived peptides (104). Their effect on placental POMC may be mediated by CRH since the addition of a CRH antagonist causes a partial block of the stimulatory effect of prostaglandins on placental POMC (104). K-opioid binding sites, for which prodynorphin-derived opioids are the main

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endogenous agonist, are also present (112), suggesting that placental dynorphins may have a local effect.

The physiological significance of placental POMC-derived peptides remains unknown. They could act either locally or systemically. There are local effects of placental ACTH which involve stimulation of the release of intrauterine prostaglandins and of the secretion of progesterone and estrogens by placenta in vitro (113). (3-endorphin has preferential affinity proposed as the prototype ligand for the e-opioid receptors identified in the rat vas deferens (114). It appears that opioids stimulate the secretion of hCG from first trimester human trophoblasts in vitro and reduce placental acetylcholine release into the fetal vessels (115). In addition, an inhibitory action of opioid peptides on placental GnRH release, similar to that found in hypothalamic GnRH neurons, has also been observed (115). Recent evidence suggests that placental steroid hormones and endogenous opioid peptides may also modulate the release of GnRH (115). Collectively, these findings suggest that the locally produced steroids and opioid peptides participate in the overall regulation of hCG secretion by the syncytiotrophoblast. The presence of receptors for estrogen, progesterone and opioid peptides in human placenta (116) supports this hypothesis.

As for the distal effects of placental POMC-derived peptides, it has been suggested that a significant percentage of maternal plasma ACTH and 3-endorphin originate in placenta. Thus, placental ACTH may participate in the regulation of the maternal hypothalamus-pituitary-adrenal axis, while placental P-endorphin may play a role in the inhibition of oxytocin, vassopressin and gonadotropin secretion, the promotion of prolactin release, and in the modulation of pain threshold during pregnancy and parturition (117).

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