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STEROID CONTROL OF GONADOTROPIN SECRETION

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Summary—Current knowledge about the mechanism and site of action of estradiol (E_2) and progesterone (P) during the menstrual cycle and the physiological role of androgens is reviewed. In normal women, the positive feedback effect of E_2 at the pituitary level is the principal event of the follicular phase inducing the LH surge. P, by its negative feedback at the hypothalamic level and by its positive feedback at the pituitary level regulates GnRH and LH secretion during the luteal phase. Androgens do not directly play a role in gonadotropin regulation.

INTRODUCTION

The role of ovarian steroid hormones on the regulation of gonadotropin secretion has been studied for many years. The present paper will summarize what we know about the mechanism and the site of action of estradiol (E_2) and progesterone (P) during the menstrual cycle. In addition, some new data about the positive feedback effect of P and the physiological role of androgens in normal women will be presented.

ESTRADIOL

The inhibitory effects of E_2 have been studied extensively [1]. E_2 represents the principal ovarian component of the classical negative feedback loop. In ovariectomized or in postmenopausal women, E_2 induces a rapid decline of circulating gonadotropin levels and reduces the amplitude of LH pulses. However, their frequency is not significantly modified. More recently, in ovariectomized rats, it has been demonstrated that E_2 negatively regulates the mRNA encoding all three gonadotropin subunits [2].

The site of the negative feedback effect of E_2 remains controversial, but a large body of evidence argues that the hypothalamus is the main site for this action. In the monkey, microinjections of E_2 into the hypothalamus are followed by a suppression of LH. In normal women, the antiestrogen clomifene citrate, which acts at the hypothalamic level, increases LH pulse frequency. It has also been reported that estrogen may regulate negatively the GnRH gene [3]. Finally, and very recently, O'Byrne *et al.* [4] demonstrated that during the late follicular phase the high plasma E_2 level was accompanied by a decrease in the hypothalamic multiunit electrical activity (MUA), and even arrest of the pulse generator.

Other studies suggest a pituitary site of action for the negative feedback effect of E_2. In the rat anterior pituitary cells, E_2 can decrease LH responsiveness to GnRH. In ovariectomized monkeys with radiofrequency lesions of the hypothalamus and treated with pulsatile GnRH administration, E_2 can reduce plasma gonadotropin levels and LH response to GnRH [1]. Thus, E_2 exerts its negative regulatory effect at both the hypothalamus and the pituitary levels.

The positive regulatory effect of E_2 on LH secretion is well known [5]. To be effective in this regard, E_2 must rise above a threshold of approx. 500 pmol/l for at least 36 h. *In vivo*, studies have clearly demonstrated that when E_2 positively regulates gonadotropin secretion, the levels of gonadotropin subunit mRNA are also positively regulated.

It is well-established that the pituitary is the main site of the positive feedback effect of E_2. This was first demonstrated in ovariectomized monkeys with hypothalamic lesions [1]. A gonadotropin surge can be obtained by E_2 administration.

Other studies suggest a hypothalamic site for the stimulatory effect of E_2. In ovariectomized ewe, Moenter *et al.* [6] reported by measuring GnRH concentrations in portal blood that the

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stimulatory action of E_2 is due to a large surge of GnRH. In women, Filicori *et al.* [7] reported an increase of LH pulse frequency concommitant with the increase of E_2 levels in the late follicular phase. These data disagree with the results recently obtained by O'Byrne *et al.* [4] that the decrease of hypothalamic MUA is coincident with the high E_2 level at that time. All these conflicting findings may be explained by some major species differences. However, there is no doubt that the increase or decrease of GnRH pulse frequency is not an obligatory signal for the LH surge. Women with GnRH deficiency exhibit ovulatory menstrual cycles during pulsatile GnRH administration at an invariant frequency.

In summary, E_2 exerts both positive and negative effects on gonadotropin synthesis and secretion and these actions are time- and dose-dependent. The consensus is that the negative feedback effect is mainly at the hypothalamic level and the positive feedback effect at the pituitary level.

PROGESTERONE

The change in the LH pattern observed in the luteal phase is produced by the feedback effect of P rather than by a timing mechanism intrinsic to the brain. The major effect of P is the decrease of LH pulse frequency. Administration of P to normal women during the follicular phase decreases LH pulse frequency from one pulse every 1 h to one pulse every 4 h. It is well-known that P acts at the hypothalamic level and inhibits GnRH pulse frequency [8]. P receptors have been recognized within the arcuate nucleus. The endogenous opiate peptides are involved in the mediation of P action. Administration of the opioid antagonist naloxone during the luteal phase increases LH pulse frequency [9].

Another action of prolonged exposure to P is its ability to prevent the LH surge induced by E_2 [10]. This effect can account for the absence of ovulation in the luteal phase of the cycle. Wildt *et al.* [11] have demonstrated in rhesus monkeys with the hypophysiotrope clamp preparation and GnRH replacement that this surge blocking effect of P could be attributed to a blockade of the pulsatile GnRH release.

Corbani *et al.* [12] reported that in ovariectomized rats after priming with E_2, P induced a decrease in the pituitary content of both α and β LH mRNAs. However, this is no evidence for a direct pituitary effect. P could decrease indirectly the stimulatory action of GnRH on LH synthesis. The positive regulatory effect of P is more controversial.

In women, the small amount of P produced by the preovulatory follicle augments the positive feedback action of E_2 and amplifies the duration of the LH surge. Similarly, during the menstrual cycle, LH pulses are less frequent but also of larger amplitude in the luteal phase than in the follicular phase. Filicori *et al.* [13] reported that the LH pulse amplitude was higher in early luteal phase and decreased by the mid-luteal phase from day 5 to day 9, decreasing further in the late luteal phase. Thus, it has been suggested that P exerted a biphasic effect on LH secretion and this effect would be dependent upon duration and plasma levels of P [14]. A relatively short exposure to a low concentration of P would have an acute facilitatory action. But, the mechanism is not clarified. Odell and Swerdloff [15] reported almost 20 years ago that P was able to induce LH and FSH release in postmenopausal women. More recently, it has been demonstrated in estrogen-treated postmenopausal women that a single dose of P (50 mg) resulted in a significant elevation in the basal LH concentration and in LH pulse amplitude without change in LH pulse frequency [16]. *In vitro*, Ortman *et al.* [17] demonstrated that P treatment of perfused rat pituitary cells leads to an acute facilitatory effect on GnRH-induced LH secretion. However, in women, no evidence has been established so far for a direct action of P on the pituitary gland.

To further study the positive feedback effect of P and its putative site at the pituitary level, we studied 6 women with hypothalamic gonadotropin deficiency. The patients were treated with E_2 17β, 2 mg/day orally, during two periods of 15 days separated by at least a 1 month wash out. GnRH was administered i.v. at a dose of 10 mg/pulse every 90 min during the last 5 days of E_2 treatment. P at a dose of 200 mg/day or a placebo were both administered, intravaginally in a cross-over randomized design during the 5 days of pulsatile GnRH therapy. Plasma levels of E_2 and P were measured every day during the 5 days of both treatments, GnRH + P or GnRH + placebo. Pulsatile LH secretion was studied by sampling every 10 min for 8 h before any treatment and on day 15 at the end of each treatment period. Plasma E_2 levels observed during the last 5 days of treatment remained relatively constant and not significantly different

in each patient at the time of pulse analysis. Furthermore, the plasma level of E_2 below 250 pmol/l was not sufficient to exert its positive feedback effect. Plasma P levels achieved with the vaginal pessaries were between 15 and 25 nmol/l during only the last 3 days of treatment. The LH pulse frequency was induced by the pulsatile exogenous GnRH administration. With the same plasma levels of E_2, the LH pulse amplitude was significantly increased by P compared with placebo.

Thus, our study with a constant GnRH pulse frequency demonstrated the pituitary site of the positive feedback action of P. The administration of P can have both inducive and suppressive effects on gonadotropin secretion and as for E_2, the nature of these effects is time- and dose-dependent. In the present study, the stimulatory effect of P occurred after a relatively short exposure to low P concentrations.

The main action of P is its negative feedback effect at the hypothalamic level decreasing the GnRH pulse frequency. It has been claimed that increased LH amplitude during the luteal phase was related to the decrease of GnRH and LH pulse frequency. We believe that this pattern is the consequence of P action at the pituitary level. This short effect may be better explained by an increase of gonadotropin release, rather than by an increase of gonadotropin synthesis.

ANDROGENS

In male, androgens negatively regulate α and β LH mRNA but have no effect on FSH β mRNA, which is regulated by inhibin [18]. Testosterone (T) and dihydrotestosterone (DHT) regulate LH secretion at the hypothalamic level by slowing down the frequency of GnRH pulses [19]. Bagatell and Bremner [20] reported that T had a direct pituitary effect suppressing gonadotropin secretion in men. However this effect is due to its metabolism into E_2 and can be suppressed by the aromatase inhibitor, testolactone.

In women, the physiological action of androgens on gonadotropin regulation has not yet been clearly established. Only, the action of pharmacological doses of androgens on gonadotropin regulation has been studied. Spinder *et al.* [21] reported the effects of androgens in female to male transsexual subjects. The initial effect of T is a significant increase in gonadotropin sensitivity to GnRH. This appears secondary to aromatization to E_2 rather than a direct T effect. Long-term T treatment results in a decrease in the mean plasma LH levels. In agonadal females, high serum T levels decrease both plasma LH levels and LH pulse frequency. Similarly, in normal women high doses of DHT infusion for 8 h decrease LH pulse frequency. Thus, when male levels of androgens are achieved in plasma, their effects on gonadotropin secretion are similar in women and men.

In normal women, the physiological action of androgens on gonadotropin regulation has not been established. To further study this action, we used a pure antiandrogen as a tool to investigate the effects of androgen suppression on the hypothalamic–pituitary axis in normal women [22].

Anandron is a nonsteroidal antiandrogen which interacts only with the androgen receptor. It does not interact with E_2 or P receptors. It has no antigonadotropic activity. Its half-life is about 45 h. Nine normally cycling women with acne and seborrhea received Anandron 100 mg twice daily and a placebo in a crossover design study for two consecutive cycles separated by one cycle. It has been previously shown that 200 mg of Anandron induces in normal men an increase in plasma T and LH levels. All the women treated were asked to use barrier methods of contraception. LH pulse frequency and amplitude were studied by sampling every 10 min for 8 h and the LH response to GnRH were determined on day 5 of each cycle. Pulse analysis was performed by the cluster program of Veldhuis and Johnson [23]. Plasma E_2, T, androstenedione (A), 3α-androstanediol glucuronide (3α-diol G) and SHBG were measured on days 5, 10, 20 and 24 of each cycle. Plasma P levels were determined on days 20 and 24 of each cycle. Plasma Anandron was measured by RIA at each cycle of treatment. All menstrual cycles remained ovulatory as assessed by basal body temperature and by P levels.

The LH pulsatile profile from the same woman on day 5 of four cycles during placebo and Anandron was similar. The amplitude and the frequency of LH pulses did not change significantly during the two cycles with Anandron and the two cycles with placebo in the 9 women studied. The LH response to GnRH was similar during Anandron or placebo administration. Plasma concentrations of all steroids E_2, T, A and 3α-diol G were not modified by Anandron when compared with placebo cycles. Anandron did not change significantly SHBG concentration. Thus, administration of

Anandron in a group of normally cycling women produced a clinical improvement of acne without any significant hormonal change. More precisely, in spite of high plasma Anandron levels, the frequency and amplitude of LH pulses remained unchanged. All these data emphasize that physiological levels of androgen have no action on the regulation of gonadotropin secretion in normal women.

In conclusion, in normal women, the positive feedback effect of E_2 at the pituitary level is the principal event of the follicular phase inducing the LH surge. P by its negative feedback at the hypothalamic level and by its positive feedback effect at the pituitary level regulates GnRH and LH secretion during the luteal phase. Androgens do not directly play a role a gonadotropin regulation.

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