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# STEROID MODULATION OF THE HYPOTHALAMIC-PITUITARY SYSTEM IN THE SECRETION OF REPRODUCTIVE HORMONES*

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## INTRODUCTION

The brain-pituitary system which controls the reproductive hormones, gonadotropins and prolactin (PRL), appears to be distinct from that of metabolic hormones—TSH, ACTH and GH. In the latter, the major process of regulation is operated through a negative feedback system of the target organ hormones or signals (ACTH-Cortisol, TSH-thyroxine and GH-glucose) with a CNS override during sleep. In addition to this reciprocal relationship between target organs and the brain-pituitary system, the regulation of gonadotropin release exhibits a striking departure in which an overlapping negative and positive feedback system are operative *via* ovarian signals—estradiol and progesterone [1, 2]. PRL, when viewed as an indispensable hormone for reproduction has no specific target organ feedback but is regulated by a signal generated by the feto-placental unit of superphysiological amounts of estrogen which preferentially promote PRL secretion [3–6] and by a central neuronal mechanism of a sleep-induced augmentation [7].

Thus, it would seem that the target organ hormones play a critical modulating role upon the brain-pituitary system. The recent availability of the synthetic hypothalamic hormones—LRF and TRF, has afforded a new tool in the delineation of functional characteristics of the steroid feedback modulation on the pituitary release of gonadotropins and PRL. This paper presents the results of our study in the inhibition and amplification of pituitary responses to TRF and LRF during estrogen and progesterone imposed experiments in normal and hypogonadal women. Serum LH, FSH, PRL, estradiol and progesterone were measured by radioimmunoassay [8–11].

### *Effects of estrogen on PRL secretion and TRF induced PRL release*

1. *Acute effect.* The acute effect of pharmacological doses of $E_2$ *via* constant infusion (50 μg/h × 4 h) was assessed in 10 hypogonadal subjects. As shown in Fig. 1, there was a significant decrease in PRL levels during the infusion, and a significant increase in PRL-release which lasted for several hours immediately following the infusion. This finding suggests that acute pharmacological doses of $E_2$ exerts both inhibitory and stimulatory action on the pituitary PRL secretion. Since under physiological circumstances, acute rise of $E_2$ to such magnitude (~1000 pg/ml) does not occur, this finding must be viewed as a pharmacological action of $E_2$ on PRL secretion and bears no physiological implication.

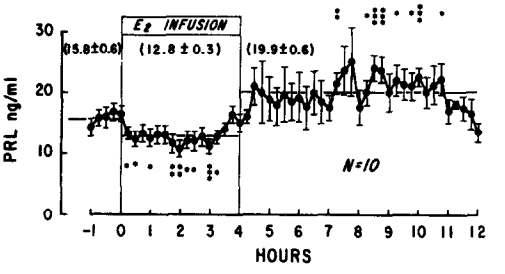


Fig. 1. Serum PRL concentrations before, during and following $E_2$ infusion determined in samples obtained at 15 min intervals in 10 hypogonadal subjects.

2. *Chronic effect.* The chronic effect of EE treatment on PRL-secretion has been assessed in hypogonadal women. Small doses of EE (1 μg/kg/day) induced a significant elevation of serum PRL levels with 1 week of treatment and a further rise until a plateau was reached in about 3–4 weeks to levels of more than 3 times of the initial concentration. The corresponding LH and FSH fell progressively to premenopausal levels at the 4th week of treatment [12]. With

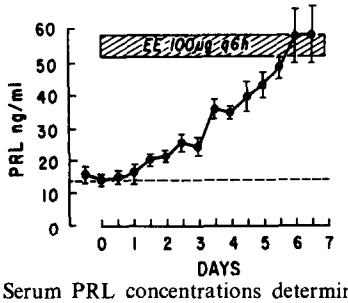


Fig. 2. Serum PRL concentrations determined at 15 min intervals before and during treatment with EE (1 μg/kg/day) in 6 experiments performed in 4 hypogonadal female subjects. The horizontal line represents the transverse mean.

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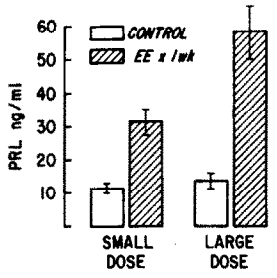


Fig. 3. The augmented PRL secretion after 1 week of EE treatment is significantly greater ($P < 0.01$) in subjects receiving large doses (400 μg/day) than in subjects receiving small doses (1 μg/kg/day) of treatment.

large doses of EE (400 μg/day), a more rapid elevation of PRL levels within 36 h after initiation of treatment was found (Fig. 2). At the end of 1 week of treatment, the rise of PRL levels was significantly greater than that found during small doses of EE treatment to 5 times the initial concentration (Fig. 3).

3. *Effect of estrogen on PRL release in response to TRF.* PRL release in response to TRF (500 μg, IV bolus) was evaluated in 6 subjects receiving chronic treatment with either premarin (1·25 mg/day) or in the form of sequential contraceptive steroids. The same TRF test was performed 4 weeks after the discontinuation of estrogen treatment. As can be seen in Fig. 4, a significantly greater TRF induced PRL release without alteration in TSH responses was found during estrogen treatment. These data confirm previous reports [13–14] and suggest a direct estrogen-lactotroph feedback. To account for the enhanced PRL-release by estrogen, hypothalamic suppression of prolactin-inhibiting factor as well as a direct stimulatory action on pituitary lactotrophs may have to be ascribed as has been demonstrated by *in vitro* experiments [3, 4, 15].

These studies represent the first clear demonstration of the augmentation of pituitary PRL secretion by estrogen in humans. Under physiological circumstances, a temporal relationship between the rise in pituitary PRL secretion and the increase in circulating estrogen can be found; such as in girls (but not in boys) during late puberty [16] and during pregnancy [6]. PRL release has also been found to be greater in females than in males under pharmacological stimuli such as arginine [17], perphenazine [13] and TRF [13, 17]. These collective observations together with our present findings clearly establish that estrogen exerts a dose-related augmentation on the pituitary PRL secretion in humans. The lack of a clear-cut elevation of PRL levels during the preovulatory phase of the menstrual cycle [6, 8] could be explained by the brief duration (3–4 days) and modest amount of endogenous estrogen which may not be sufficient to induce an amplification effect on the pituitary PRL secretion.

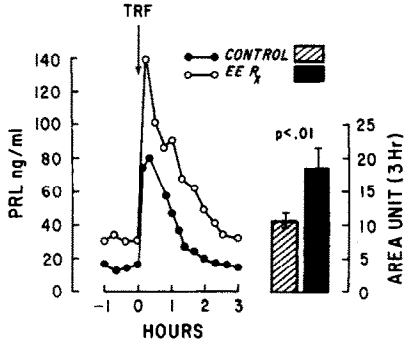


Fig. 4. Pituitary PRL-responses to TRF (500 μg) with or without estrogen treatment. The quantitative secretion (area under the curve) is significantly greater during estrogen treatment.

*Effects of gonadal steroids on the pituitary gonadotropin responsiveness to LRF*

Our early attempt to test pituitary "sensitivity" to synthetic LRF (150 μg) during different phases of the menstrual cycle [18] revealed that a greater and more sustained LH and FSH release from the early to late follicular phase of the cycle and a "window" of maximal sensitivity to LRF for both LH and FSH release occurs at midcycle. We have suggested that this apparent increase in pituitary sensitivity to LRF stimulation is a result of the feedback action of progressively increasing levels of circulating estradiol. Since a greater response was also found during the mid-luteal phase of the cycle, the possibility of an additive and synergistic action of progesterone in the augmentation of pituitary responsiveness to LRF was proposed and subsequently elucidated. The effects of acute and chronic estrogen administration on the pituitary response to LRF were first studied in hypogonadal subjects. A rapid increase of circulating estradiol concentrations to levels of 700–900 pg/ml achieved by constant infusion (50 μg/h × 6 h) induced a prompt and marked inhibition of gonadotropin release in response to LRF [19]. Since under physiological circumstances, an acute rise of $E_2$ to such magnitude (~900 pg/ml) does not occur, this finding bears little or no significance in the homeostatic gonadotropin regulation but it does imply a demonstration of the pituitary component of the negative feedback system of $E_2$ on gonadotropin output. When chronic and uninterrupted administration of small dose of ethinyl estradiol (EE, 1 μg/kg/day) was assessed at weekly intervals during treatment, the pituitary LH release to LRF showed a biphasic course of response: an initial augmentation (during the first two weeks) followed by progressive inhibition. During these serial studies, FSH-release was perferentially blunted [19]. Studies in subjects receiving oral contraceptives reveal a remarkable augmentation of pituitary responsiveness to LRF for the release of LH but not for FSH, evident only in subjects receiving sequential estrogen-progestin regimen [20]. Taken together, these earlier findings support the concept that changes in pituitary sensitivity to LRF during the menstrual cycle are in part determined by estradiol levels. More direct evidence is afforded by our recent demonstration (Fig. 5) that the usually aug-

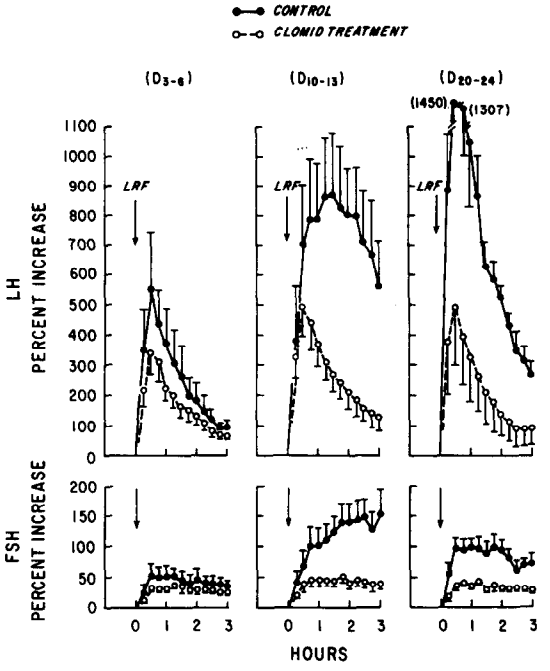


Fig. 5. Effects of clomiphene treatment (100 mg/day × 5 days) on pituitary response to LRF (150 μg) during three different phases of the menstrual cycle as compared in the same group of subjects without clomiphene treatment.

mented pituitary gonadotropin response to LRF, seen during high estrogen phases of the ovulatory cycle is completely eliminated by the administration of an anti-estrogen, clomiphene [21]. With the Clomid treatment, the pituitary response to LRF during three different phases of the menstrual cycle remains qualitatively and quantitatively constant and resembles the LRF responsiveness of the male pituitary.

The ability of progesterone to trigger an acute release of gonadotropins in the estrogen primed hypogonadal women has been reported [22–25], but evidence that this effect may have physiologic significance during the menstrual cycle is lacking. To approach this question, we have administered a low dose of progesterone (10 mg, i.m.), to normal cycling women at various times during the follicular phase (Fig. 6). A marked, but brief, surge of both LH and FSH was elicited in the late follicular but not during the mid-follicular phase of the menstrual cycle. Thus, this facilitory action of progesterone on the release of gonadotropins appears to be dependent on relatively high circulating levels of estradiol and is functional at relatively low circulating progesterone levels. Since it has been demonstrated that a 3-fold increase in progesterone concentration (from 0·5 to 1·5 ng/ml) occurs at the time of midcycle gonadotropin surge [26, 27], a synergistic role of progesterone in the amplification of this estrogen initiated surge should be considered.

*Experiments utilizing pulses of LRF*

In an attempt to more closely approximate the hypothalamic input either as episodic or constant delivery of LRF to the adenohypophysis, an experimental design using pulses of LRF (five, serial 10 μg doses at 2 h intervals) was implemented. It is reasoned that these simulated hypothalamic inputs may disclose qualitative and quantitative aspects of the pituitary sensitivity and reserve which cannot be assessed by a single large dose of LRF. The immediate release of pituitary gonadotropin in response to a small increment of LRF may be regarded as an estimation of pituitary sensitivity; since this stimulus (small pulse of LRF) is sufficiently brief, it is likely that the rapidity

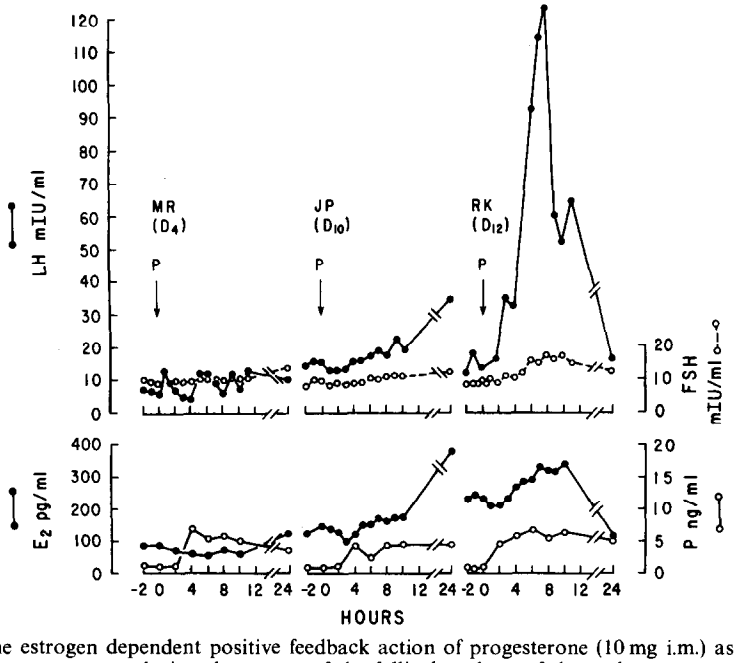


Fig. 6. The estrogen dependent positive feedback action of progesterone (10 mg i.m.) as demonstrated during the course of the follicular phase of the cycle.

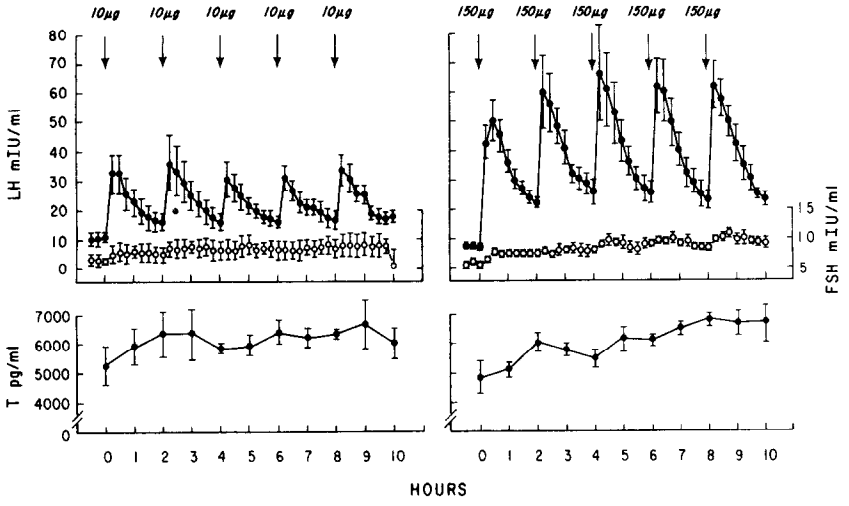


Fig. 7a. Pituitary gonadotropin responses to a large dose (150 μg) and a small dose (10 μg) pulses of LRF at two hourly intervals studied in 4 normal adult male (testosterone concentrations determined at hourly intervals are also shown).

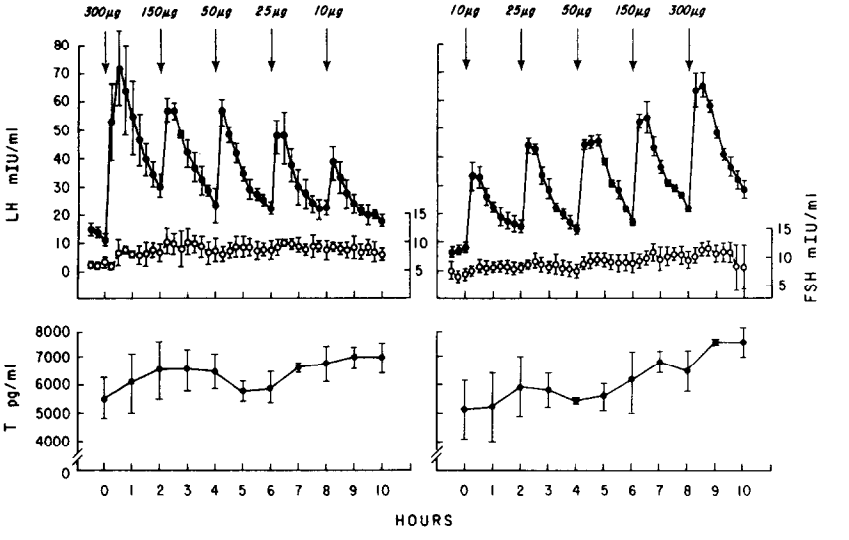


Fig. 7b. Pituitary gonadotropin responses to an incremental and decremental pulses of LRF studied in 4 normal adults (testosterone concentrations are also shown).

and quantity of initial release is reflecting the readily releasable pool of gonadotropin in the pituitary. Subsequent stimulation by repeat bolus-injections may elicit the release of stored gonadotropin—the ultimate releaseable pool—which may represent the pituitary reserve.

1. *Normal men.* In response to constant LRF pulses (Fig. 7a), the pattern of gonadotropin release in eugonadal men is qualitatively similar, but quantitatively greater than the spontaneous pulsatile pattern observed in normal adult men [28]. Incremental and decremental doses of LRF pulses were followed by corresponding changes in the pituitary release of LH (Fig. 7b). It is apparent that a small increase or decrease in LRF input can be recognized by the pituitary gonadotrophs of the male pituitary and these findings provide the physiological basis for our subsequent studies. FSH response in these studies were small and testosterone levels were not significantly changed.

2. *Normal women.* The response to LRF pulses throughout the menstrual cycle is illustrated in Fig. 8. In each instance, circulating estradiol and progesterone are recorded concomitantly with the change in gonadotropin levels. Subject K.G. was studied at the time of the midcycle surge as indicated by the high baseline LH and FSH levels. During the early follicular phase, the gonadotropin response to pulses of LRF are qualitatively and quantitatively similar to those of the normal male. There were only slight increases in estradiol levels during the ten hour study periods. With increasing levels of estradiol a gradual increase in pituitary sensitivity, from the early to late follicular phase of the cycle, was observed. In contrast to the early follicular phase, a significant rise in circulating estradiol levels during the 10 h experiments

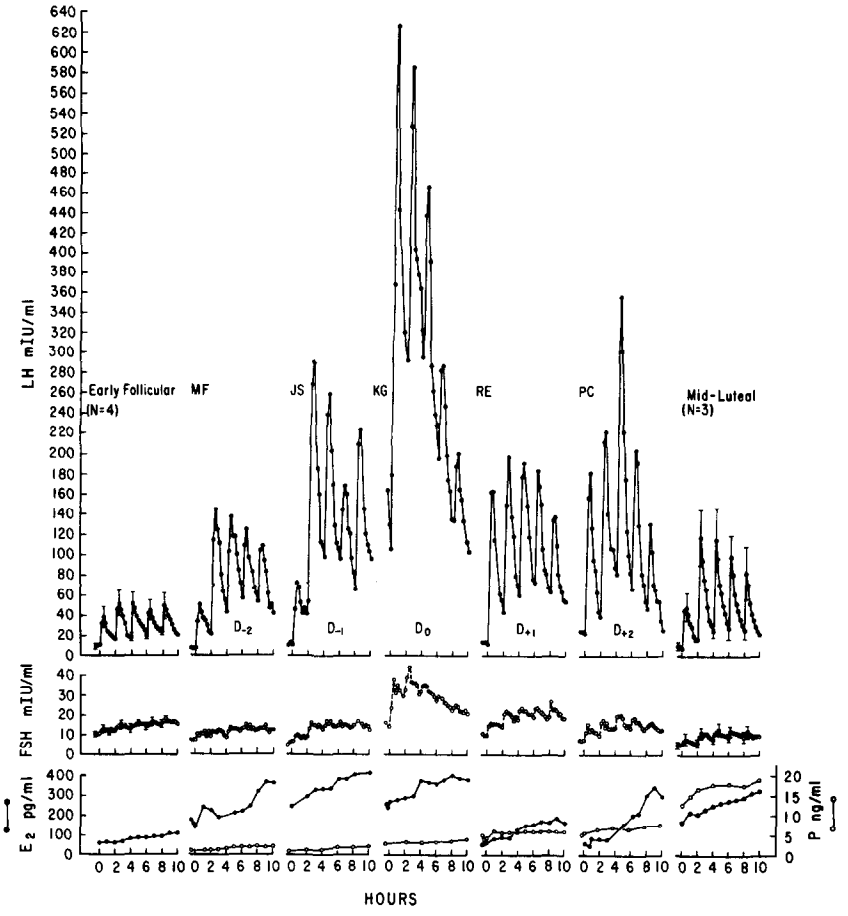


Fig. 8. The release of LH and FSH in response to "pulses of LRF" during various phases of the cycle. (Each LH spike represents response to a single LRF injection). Circulating estradiol ($E_2$) and progesterone concentrations are also depicted. (Data are centered around subject K.G. on whom the study was performed at the midcycle surge).

were seen in subjects studied at this time. The LH responses to the second and subsequent LRF pulses are markedly augmented above that of the first response. This pattern is evident only during high estrogen phases of the cycle (except midcycle surge) and thus, it may reflect an estrogen induced LRF "self-priming" phenomena of LRF action. These data suggest that incremental changes of circulating estradiol not only heighten the pituitary sensitivity to LRF but also increased the pituitary gonadotropin reserve.

Extraordinary pituitary sensitivity was observed on the day of the midcycle surge (subject K.G.) and gradually declined over the next 2 days. During the midcycle gonadotropin surge and the two days following, the quantitative response to the first LRF pulse is much greater than the initial response observed in any other phase of the cycle. This marked and rapid increase of pituitary sensitivity to LRF at midcycle, approximately twelve-fold that of the early follicular, may represent the critical event which leads to the spontaneous gonadotropin surge. Thus, it may be rationalized that the addition of small increments of endogenous LRF at this time would result in a massive discharge of gonadotropins by the pituitary.

High levels of estradiol and progesterone during the midluteal phase were accompanied by a response to LRF pulses that were qualitatively similar to that of the late follicular phase. The elevation of progesterone levels observed during the ten hour study period in the midluteal phase was not found in the early luteal phase; suggesting a shift of steroidogenic potential between early and well established luteal tissue.

3. *Effect of gonadal steroids on the modulation of pituitary sensitivity and reserve.* In order to provide direct evidence that estradiol and progesterone modify pituitary sensitivity and reserve, twice daily injections of estradiol benzoate of 2, 4, 6 and 8 $\mu$g/kg were administered to subjects during the early follicular phase of the cycle; a design which simulates the incremental circulating estradiol levels of the late follicular phase. In a similar experiment, 10 mg progesterone (i.m.) were administered at the time of the last estradiol benzoate injection. In both studies, pituitary sensitivity and reserve were evaluated at the end of treatment with the pulses of LRF described in the foregoing experiments. When compared to the response observed in the early follicular phase of the cycle, estradiol alone enhanced both pituitary sensi-

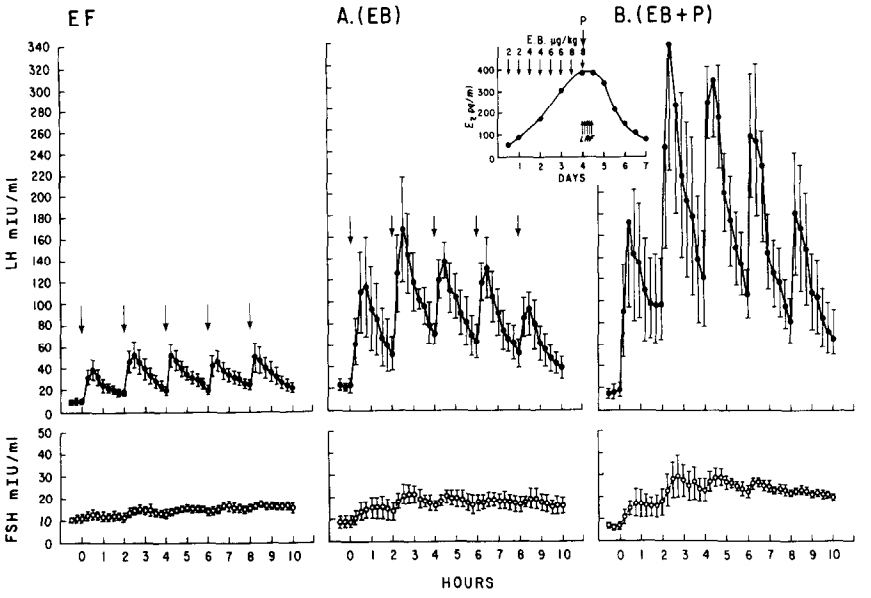


Fig. 9. The augmentation of pituitary responses to "pulses of LRF" following estradiol benzoate (EB) and EB plus progesterone administrations to subjects during their early follicular phase of the menstrual cycle as compared with responses to the same stimulus in early follicular phase (EF) without treatment.

tivity and reserve involving LH as well as FSH. A further augmentation of pituitary FSH and LH response was observed with the addition of progesterone (Fig. 9). In the absence of a feedback loop, as in hypogonadal subjects, the pituitary augmentation by estrogen was also operable (Fig. 10). But this amplification of pituitary response to LRF by estrogen involved only the reserve but not the sensitivity. The latter appears to be blunted. These data clearly establish that the pituitary sensitivity and reserve are modulated by estrogen and progesterone.

## SUMMARY AND CONCLUSION

Characterization of gonadal steroid modulation of the hypothalamic-pituitary system in the release of gonadotropin and PRL was made. The pituitary gland represents a major feedback site for the regulation of optimal delivery of tropic hormones of reproduction via steroid signals; estrogen provides a time and dose related positive feedback loop in the preferential augmentation of pituitary PRL secretion; based on changes in circulating levels, the overlapping negative and positive feedback effect of estrogen on gonadotropin output can now be meaningfully correlated with the estrogen directed changes in pituitary dynamics—a time and dose related increase in pituitary sensitivity and reserve. Progesterone in low concentration has a facilitory action on the pituitary response to LRF operative only with prior exposure to an optimal amount and duration of estrogen. A high concentration of progesterone, as in midluteal

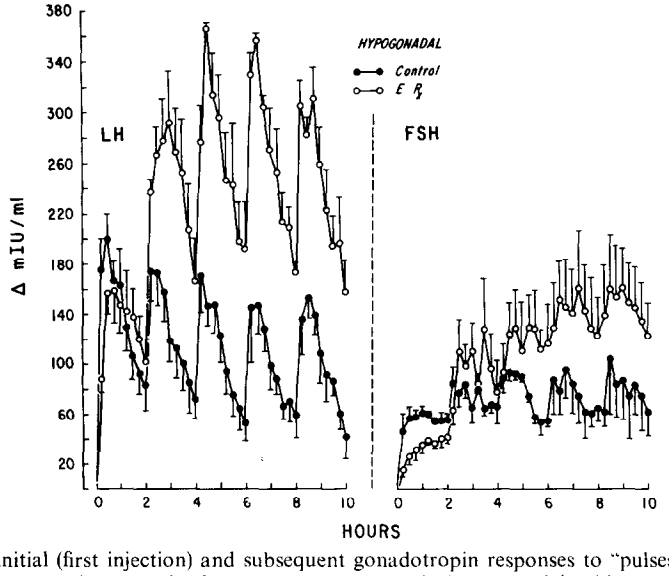


Fig. 10. The initial (first injection) and subsequent gonadotropin responses to "pulses of LRF" before and one week after estrogen treatment in hypogonadal subjects.

phase tends to attenuate the estrogen augmentated pituitary response to LRF.

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