Journal of Steroid Biochemistry, 1975, Vol. 6, pp. 1061-1066. Pergamon Press. Printed in Great Britain.

THE EFFECT OF SEX STEROIDS ON PITUITARY RESPONSIVENESS TO GONADOTROPIN RELEASING HORMONE

ALIZA ESHKOL, B. LUNENFELD* and V. INSLER†

Institute of Endocrinology, the Chaim Sheba Medical Center, Tel-Hashomer, Israel

SUMMARY

Gonadotropin secretion is controlled mainly by feedback effects of gonadal steroids at hypothalamic sites. The existence of a regulatory role of gonadal steroids at the pituitary has also been suggested. Results obtained with exogenous steroids as reflected by pituitary responsiveness to GnRH are, however, contradictory. Discrepancies can be attributed to types of steroids administered, doses used and the impossibility to distinguish between the effect on the pituitary from that on the hypothalamus. To overcome the above limitations, the effect of endogenous steroids on pituitary response to a standard dose of GnRH (Ayerst) was investigated in primary amenorrheic patients with isolated hypothalamic failure. The effect of GnRH was investigated under various steroidal environments obtained during cycles induced by exogenous gonadotropins. In the first phase (in the absence of ovarian steroids) GnRH evoked an increase in both LH and FSH. In the second phase, when the endogenous level of estradiol was high, GnRH did not induce an FSH release. Elevation of LH secretion began at the same time as in the first phase but was prolonged and reached higher values—coinciding with the first observed increase in plasma progesterone. In the third phase, the luteal phase (high estrogen and progesterone levels), GnRH failed to elicit either LH or FSH release. It seems therefore that estrogens sensitize the pituitary to respond to GnRH with a selective augmented LH secretion. Steroidal environment of the luteal phase diminishes the pituitary responsiveness to GnRH with respect to both FSH and LH release. It can thus be postulated that steroids can modulate pituitary responsiveness to hypothalamic stimuli.

INTRODUCTION

In the last decade great advances have been made towards the elucidation of regulatory mechanisms in reproduction and particularly in hormonal secretion.

As a result of the development of specific and sensitive radioligand assays simultaneous accurate measurements of pituitary hormones and gonadal steroids in plasma have been made. These could thus be performed at frequent intervals in normal physiological conditions as well as in various exogenously manipulated environments. A further major step toward the understanding of the dynamic relationships between the various components of the hypothalamic-pituitary-gonadal axis has been provided by the elucidation of the structure of hypothalamic gonadotropin releasing hormone (GnRH) and its subsequent synthesis. Nevertheless, the complex dynamic hormonal interplay in the human has not yet been fully elucidated. Studies aiming towards the understanding of feedback control mechanisms in the human are progressing at a slow pace mainly due to the inherent limitations in human experimentation and the almost impossible task to obtain glands from normal human subjects, isolated in a viable form, for studies under controlled conditions. Thus, any discussion on causal linkages has to rely partly on information obtained in various animal species, bearing in mind the danger of extrapolation of animal data to findings in the human. One could, however, choose patients with errors of nature who might serve as suitable models to study the pituitary response under controlled hormonal environments.

Both approaches were utilized in the present study. The direct effect of steroids on rat pituitaries as reflected by their response to GnRH was investigated *in vitro*.

Furthermore, the modulating effect of steroids was studied in women with primary amenorrhea due to hypothalamic failure. In these cases the pituitary was considered as not being regulated by endogenous gonadotropin releasing hormone(s) and any steroidal effects could be ascribed as being exerted directly on the pituitary gland and not mediated *via* the hypothalamus.

The ovaries of these patients were however capable of steroid secretion when stimulated with exogenous gonadotropins and the response to GnRH was evaluated prior to and during different phases of ovarian stimulation.

Experimental design and results

(1) Effect of steroids *in vitro* on rat pituitaries.

Hemipituitaries of adult male rats were incubated in Krebs–Ringer bicarbonate–glucose medium under a 95% O_2–5% CO_2 atmosphere. In the initial stages

* Established investigator of the Chief Scientist's Bureau, Ministry of Health.

† Dept. of Obst. and Gyn. The Chaim Sheba Medical Center, Tel-Hashomer.

Table 1. Effect of steroids on LH and FSH release *in vitro* (per mg pituitary tissue)

	LHng/mg	FSHng/mg
Control	1018	755
E_2 5 × 10^{-10}M	1021	668
E_2 5 × 10^{-8}M	1070	674
P 3·3 × 10^{-9}M	1018	738
P 3·3 × 10^{-8}M	1241	824
E_2 5 × 10^{-10}M + P 3·3 × 10^{-9}M	1121	821

of the incubation high amounts of LH* and FSH* leaked into the medium, declining later. Therefore, a preincubation of 90 min with an intermediate change of the medium at 45 min was performed. Then the medium was exchanged again and the various steroids were added (except to the controls) and incubation proceeded for 90 min (second preincubation). During this period similar amounts of LH and FSH accumulated in the control flasks and in those containing the various steroids (Table 1).

At the end of the "second preincubation" the medium was exchanged and contained in addition to the various steroids 1 ng synthetic GnRH (Ayerst) per ml medium. The GnRH caused a two-fold increase in both LH and FSH into the medium within 4 h (Figs. 1 and 2, column 2). The effect of estradiol depended on the dose added. In the presence of 5 × 10^{-8}M estradiol there was practically no response to the GnRH stimulation (Figs. 1 and 2, column 3) whereas a concentration of 5 × 10^{-10}M of estradiol significantly augmented the response of both the LH and FSH release ($P < 0.05$ for both hormones) (Figs. 1 and 2, column 4).

* Determined by the double antibody radioimmunoassay using reagents supplied kindly by Dr. A. Parlow (Rat Pituitary Hormone Distribution Program, NIH).

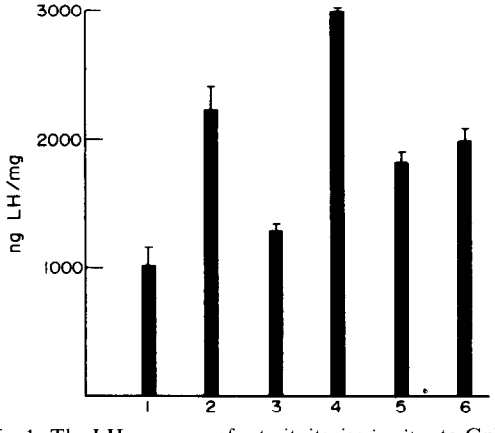

Fig. 1. The LH response of rat pituitaries *in vitro* to GnRH alone and in the presence of 17β-estradiol (E_2) and progesterone (P).
Legend: 1. Control without GnRH; 2. 1 ng GnRH/ml medium; 3. to 6. 1 ng GnRH/ml medium in the presence of: 3. $E_2$5 × 10^{-8}M; 4. $E_2$5 × 10^{-10}M; 5. $E_2$5 × 10^{-10}M + P 3·3 × 10^{-9}M; 6. P 3·3 × 10^{-9}M.

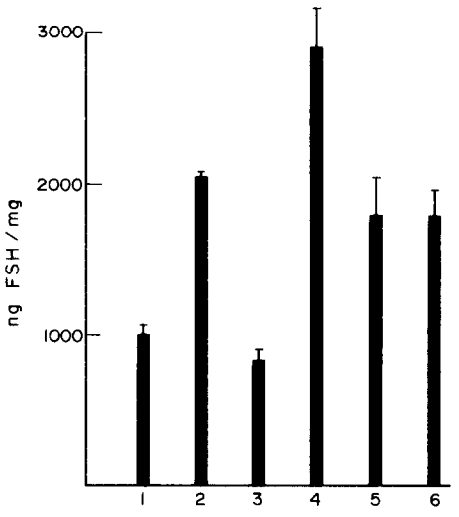

Fig. 2. The FSH response of rat pituitaries *in vitro* to GnRH alone and in the presence of 17β-estradiol (E_2) and progesterone (P).
Legend: As in Fig. 1.

In the presence of 3·3 × 10^{-9}M progesterone the response to GnRH was unaffected *i.e.* a two-fold increase in FSH and LH was observed (Figs. 1 and 2, column 6). However, in the presence of progesterone together with a low concentration of estradiol the augmentory effect of estradiol alone was abolished (Figs. 1 and 2, column 5). A ten-fold concentration of progesterone (3·3 × 10^{-8}M) did also not affect the LH and FSH secreted as a response to GnRH.

(2) Effect of various phases of induced ovarian function in amenorrheic patients on the response to GnRH.

All the patients who participated in this study had primary amenorrhea. They were of normal female phenotype and karyotype. Their basal levels of estrogens, LH and FSH are listed in Table 2. Methods employed for hormone assays were described previously [1]. The lack of estrogenic activity in all patients was further evident from the lack of occurrence of withdrawal bleeding following administration of medroxy-progesterone-acetate (MAP).

Dynamic stimulatory tests were performed in order to localize the origin of the hormonal insufficiency and to exclude pituitary and ovarian unresponsiveness to the appropriate stimuli. The approach to this is illustrated schematically in Fig. 3. None of the

Table 2. Age, karyotype and baseline levels of hormones

Patient	A.V.	G.S.	Z.A.	B.E.
Age	30	27	29	22
Karyotype	46XX	46XX	46XX	46XX
Baseline levels				
total urinary estrogens μg/24 hours	<10	<10	<10	<10
Plasma LH mIU/ml	2·5	2·9	1·1	1·8
Plasma FSH mIU/ml	4·2	3·9	<0·5	0·7

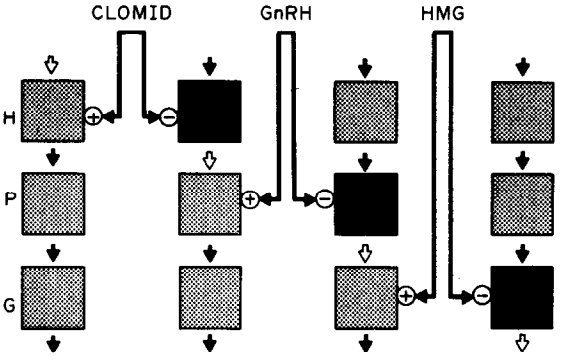

Fig. 3. Schematic illustration of stimulation tests used in amenorrheic patients to establish the functional capacities of the hypothalamus (H), the pituitary (P) and the ovary (G).

patients responded to stimulation with clomiphene citrate but all had a pituitary response to GnRH and an ovarian response to stimulation with human menopausal gonadotropins (HMG) (Table 3). The response to GnRH was not uniform in the 4 patients (Table 4).

Patient Z. A. whose initial LH response to GnRH was only marginal with no measurable increase in FSH, was capable of secreting significantly higher amounts of both hormones after 1 daily injection of GnRH for several days (Table 5).

All four patients received GnRH prior to ovarian stimulation *i.e.* in the absence of measurable amounts of estrogens and progesterone (Phase I) and after ovarian stimulation with HMG (Phase II), except for patient A.V. who received pregnant mare serum gonadotrophin (PMSG) which does not cross react in the radioimmunoassay with LH and FSH. GnRH was given when estrogen levels were elevated to "preovulatory" levels whereas progesterone was undetectable.

GnRH was given in all instances in a single dose of 100 μg i.m. except for A.V. who received in all phases an infusion of 500 μg over a period of 20 min.

Table 3. Response to various dynamic stimulation tests

		A.V.	G.S.	Z.A.	B.E.
Response to Clomiphene		—	—	—	—
GnRH	FSH	+	+	+*	+
	LH	+	+	+	+
HMG		+	+	+	+

* only after repeated GnRH stimulation.

Table 4. Maximal rise of LH and FSH after single GnRH stimulation (mIU/ml)

		A.V.	G.S.	Z.A.	B.E.
LH	Control	2·5	2·9	1·1	1·8
	GnRH	12·2	15·7	1·8	2·7
FSH	Control	4·2	3·9	<0·5	0·7
	GnRH	9·0	13·0	<0·5	1·8

Table 5. FSH and LH secretion in response to GnRH on the first day of administration and on the 13th day following one daily injection of GnRH (patient Z.A.)

Time (min.)	FSH Day 1	FSH Day 13	LH Day 1	LH Day 13
0	<1·5	<1·5	<1·2	<1·2
15	<1·5	2·0	1·6	4·9
30	<1·5	—	2·0	—
60	—	3·6	—	9·6
90	<1·5	2·9	2·0	9·4
120	<1·5	2·9	1·5	5·8
180	<1·5	3·1	<1·2	4·4
24 hr.	<1·5	<1·5	<1·2	<1·2

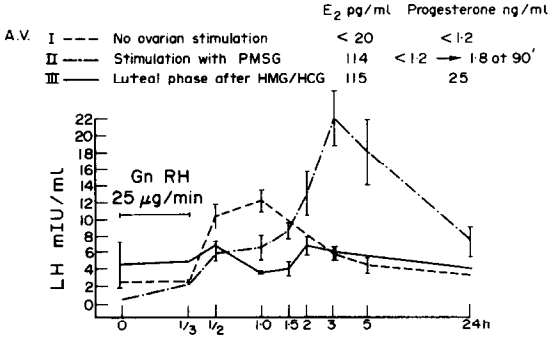

Fig. 4. The LH response of patient A.V. to GnRH infusion prior to ovarian stimulation (Phase I), after ovarian stimulation with PMSG (Phase II) and in the luteal phase (III) after induction of ovulation with HMG/HCG.

In patients A.V. and G.S. the LH response in Phase II was significantly higher and of longer duration than in Phase I (Figs. 4 and 5). In the presence of the elevated endogenous estrogen levels there was no FSH response to GnRH in patient A.V. (Fig. 6) and a diminished response in patient G.S. (Fig. 7). The elevated control level of FSH in this patient in phase II was due to residual FSH from the treatment with HMG.

Patients A.V. and G.S. received an additional treatment course with HMG and when follicular maturation was presumed to be adequate, ovulation was induced with HCG. When plasma progesterone

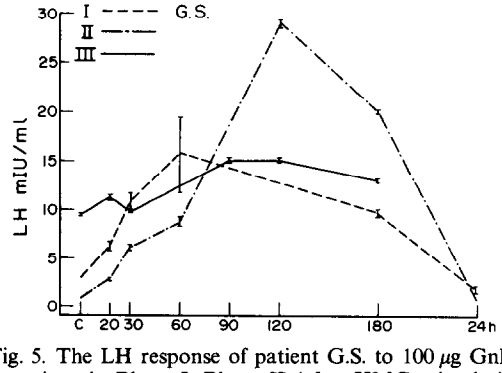

Fig. 5. The LH response of patient G.S. to 100 μg GnRH i.m. given in Phase I, Phase II (after HMG stimulation) and Phase III (luteal—after induction of ovulation with HMG/HCG).

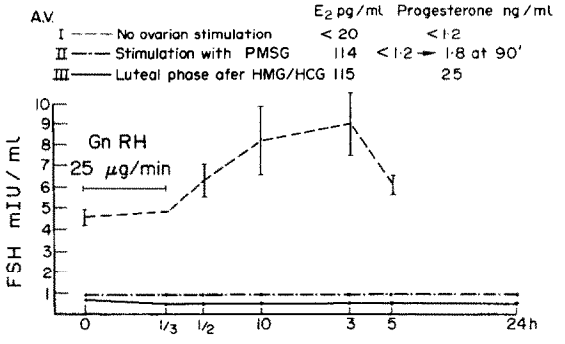

Fig. 6. The FSH response in patient A.V. to GnRH infusion in 3 phases as described for Fig. 4.

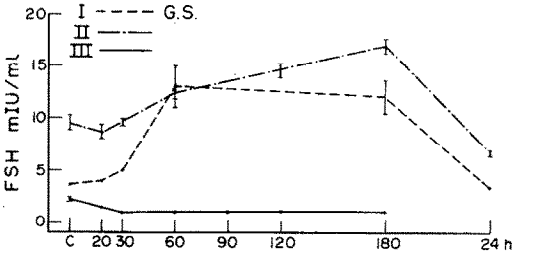

Fig. 7. The FSH response of patient G.S. to 100 μg GnRH i.m. in 3 phases as described for Fig. 5.

reached the level of 25 ng/ml and 20 ng/ml respectively, an additional GnRH stimulation was performed (Phase III).

During this phase (high estrogens and progesterone levels) no significant elevations of neither LH nor FSH were induced (Figs. 4–7).

In patients Z.A. and B.E. who had low base levels of LH and FSH, the response to GnRH prior to ovarian stimulation (Phase I) was only marginal. In patient Z.A. the response increased upon repeated administrations of GnRH (Table 5). There was no increase in estrogen secretion during the period of daily GnRH administration. Following ovarian stimulation with HMG (Phase II), when urinary estrogen levels were 211 μg/24 h (Z.A.) and 125 μg/24 h, no significant change of the LH response to GnRH was observed (Fig. 8). However, the FSH response was significantly augmented as compared to

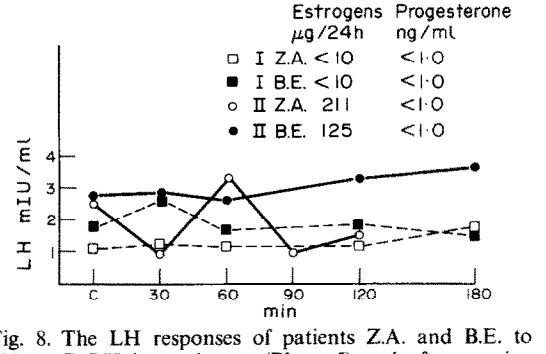

Fig. 8. The LH responses of patients Z.A. and B.E. to 100 μg GnRH i.m. prior to (Phase I) and after ovarian stimulation (Phase II).

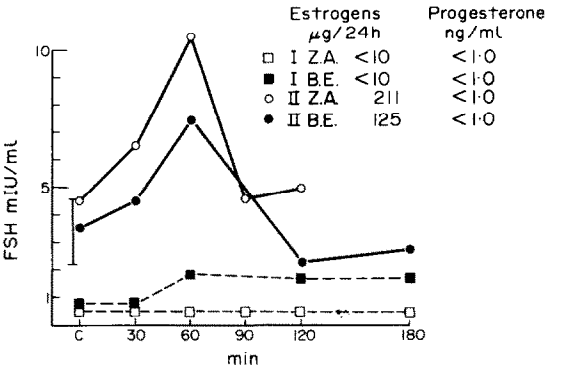

Fig. 9. The FSH responses of patients Z.A. and B.E. to 100 μg GnRH i.m. in Phases I and II.

Phase I (Fig. 9). The elevated control values of FSH prior to GnRH administration in Phase II are due to residual FSH from the HMG treatment.

DISCUSSION

Evidences are continually accumulating to indicate that feedback mechanisms controlled by steroids are being exerted at both the pituitary and hypothalamic level [2–4].

To evaluate the direct effect of sex steroids on the pituitary the *in vitro* studies were chosen. Estradiol had in the study described here (static incubation) a dual effect. Exposure of the pituitaries to a concentration of 5×10^{-10} M E_2 caused a two-fold higher increase of LH (over control levels) in response to the GnRH. Estradiol in a concentration of 5×10^{-8} M completely inhibited any LH response of the pituitaries to the GnRH stimulation. The latter findings are in accord with the observation that E_2 in a concentration of $1{\cdot}2 \times 10^{-7}$ M to $1{\cdot}7 \times 10^{-6}$ M inhibited the response to GnRH [5]. Estradiol alone (without stimulation with GnRH) had however no effect on LH or FSH release in any of the concentrations. Schally *et al.*[5] found that LH secretion was below control levels in the presence of E_2. The discrepancy between these findings is probably due to the higher doses of E_2 employed in their studies *i.e.* $3{\cdot}4 \times 10^{-6}$M–$3{\cdot}4 \times 10^{-5}$M. The augmentory effect of E_2 (in physiological doses 5×10^{-10} M) on FSH release was less pronounced but statistically significant.

Progesterone in a concentration of $3{\cdot}3 \times 10^{-9}$ M had no effect neither on the base level secretion of either LH or FSH nor on the stimulatory effect of GnRH. The addition of this amount of progesterone to estradiol however counteracted the sensitizing effect of estradiol. In the studies of Schally *et al.*[5] complete inhibition of gonadotropins *in vitro* by these steroids has been reported and the combined effect of the two steroids was more pronounced than of each of them separately; the doses were, however, higher (E_2 $3{\cdot}3 \times 10^{-6}$ M and progesterone $3{\cdot}3 \times 10^{-5}$ M).

It is thus clear that steroids modulate the pituitary sensitivity to stimulation by GnRH. The effect is however dose dependent and estradiol, as found during normal physiological conditions *i.e.* in the range of 10^{-9} M to 10^{-10} M, causes an augmented release of LH and FSH but only in the presence of GnRH. Significantly higher doses of estradiol *i.e.* in the range of 10^{-7} M to 10^{-5} M have an opposite effect on the pituitary. Progesterone alone, at least in our studies, did not affect pituitary responsiveness to GnRH but did, however, abolish the sensitizing effect of low doses of estradiol. Data on the changing pattern of pituitary sensitivity to GnRH in relation to the cycle phase are somewhat conflicting. It has been shown by several investigators that administration of GnRH in midcycle in the human resulted in an augmented release of both FSH and LH as compared to early follicular and mid luteal phase. The existence of a causal relationship between estrogens and LH surge has been elegantly illustrated by Ferin *et al.*[6], in the monkey. These authors showed that by administering diethylstilbesterol (DES) to monkeys immunized against E_2 (and who became anovulatory) an LH surge was induced within 36 h. This effect is however again a combined action at both the pituitary and hypothalamic levels. The latter is indicated by the fact that the early effect of DES (within 2 h) was a lowering of plasma LH levels. Administration of 17β-estradiol to normal women on the second day of the menstrual cycle diminished the response to GnRH [7].

It has also been demonstrated that the effectiveness of estrogen administration to induce gonadotropin release depends on the stage of the menstrual cycle [8].

In studies conducted in normal subjects the site of this modulating effect of ovarian steroids could not be localized since the end result *i.e.* the elevation of plasma gonadotropins represents the sum total of an effect on the whole hypothalamic-pituitary axis. The experimental design in the human subjects of this study was chosen in order to assess the modulating effect of endogenous ovarian steroids at the pituitary level in patients with impaired hypothalamic function. Moreover, an attempt was made to study whether steroids can exert an action on pituitaries with diminished or impaired capacity to respond to GnRH.

The two initially "unresponsive" patients in our study did not respond to GnRH stimulation with an elevation in LH even in the presence of high endogenous estrogens. The steroids however affected in these patients the pituitary capacity to release FSH in response to GnRH (Fig. 9). Schneider *et al.*[9] have shown that some amenorrheic patients initially unresponsive to GnRH did respond with LH release to the same stimulus when pretreated with estrogens. These results clearly indicate that in such cases of "hypothalamic failure" estrogens might facilitate GnRH action by increasing pituitary sensitivity, but the effect is not uniform and no generalized conclusions can be drawn. One of the two initially "unresponsive" patients started however to respond to GnRH by both LH and FSH release after daily administration of GnRH for 13 days. Several patients with a similar effect of repeated GnRH stimulation (though only LH was determined) were described by Schneider *et al.*[9].

Diminished responsiveness to GnRH in several physiological conditions has also been observed. In the ewe during pregnancy the FSH response to GnRH decreased gradually. At 18 weeks of gestation, around parturition and 3 weeks post partum there was no response to GnRH. Signs of a recovery of response were observed at 6 weeks [10]. There was also no LH or FSH response to GnRH in women 7–10 days and even 3 weeks post partum but by 6 weeks the response of both hormones to GnRH was restored [11–12]. Jacobs and Jequier[13] reported that women taking combined ethynyl estradiol plus progestogen had impaired LH and FSH responses to GnRH. These observations can be interpreted as being a result of a relative insensitivity of the pituitary as a result of prolonged deprivation of GnRH due to steroid suppression of the hypothalamus or pituitary desensitization by prolonged exposure to high doses of estrogens and progesterone, or due to both mechanisms.

To evaluate the selective effect of steroids on the pituitary, patients (A.V. & G.S.) exhibiting a normal pituitary responsiveness to a single administration of GnRH were chosen. In these patients GnRH induced an augmented release of LH in the presence of elevated endogenous estrogens (Figs. 4, 5). Since the hypogonadotropic state of these patients originated from a hypothalamic insufficiency, the augmented LH response can be attributed to an effect of the estrogens on the hypophysis resulting in an increased sensitivity to the administered GnRH.

The lack of LH release in response to GnRH when both estrogens and progesterone were elevated (Phase III) is in contradiction to findings in normal women during the luteal phase [14–15]. It might be argued that in normal women in whom the pituitary is cyclically stimulated by the hypothalamus, the pituitary is less sensitive to the inhibitory effects of steroids. Furthermore, it is also possible that the administered GnRH acts in concert with endogenous hypothalamic hormone and that under such an increased stimulation the inhibitory effect of both estrogens and progesterone are partially overcome. In the two patients with adequate FSH and LH responses to GnRH stimulation, an elevated estrogen level (after gonadotropin stimulation-Phase II) diminished the FSH response to GnRH. In normal subjects GnRH in the late follicular phase, is, however, effective in stimulating FSH release [14–15]. Also in the normal menstrual cycle, midcycle elevations of FSH have been repeatedly reported (Cargille *et al.*[16–17]. Vande Wiele *et al.*[18] postulated that the midcycle elevation of FSH is brought about by a negative feedback of estradiol which declines before or during the LH midcycle peak.

Leyendecker *et al.*[20] observed that the midcycle LH peak is actually composed of two peaks and only the first of them is accompanied by an FSH elevation. Moreover, these authors showed that the second LH peak appears after a decline in estradiol levels. If these observations will be recurrently found in more subjects it could be postulated that the first LH peak is induced by the action of the elevated estrogens on the pituitary which becomes more sensitive and thus releases more LH without necessitating an elevation in GnRH. This increased plasma LH influences the steroid biosynthetic pathways of the preovulatory follicle resulting in an increase of C_{21}/C_{18} steroid ratio and a temporary decrease in estradiol. This estrogen decline might then trigger an increase of secretion of releasing hormone which induces an elevation of both FSH and LH.

Alternatively, a positive feedback mechanism mediated by the initial increase of progesterone might be operative [19–20]. This hypothesis is supported by the observation that progesterone administration in estrogen pretreated women induced a significant elevation of LH and FSH. This effect was demonstrated to be dose dependent and upon increase of plasma progesterone levels a negative feedback mechanism became operative. The midcycle rise of LH and FSH and their subsequent decline are thus a result of a delicate balance between: the modulating effect of estrogens in increasing pituitary response to GnRH; the positive effect of the changing ratio of progesterone/estrogens at the hypothalamic level; the negative effects of estrogens at the hypothalamic level; the synergistic suppressive effect of progesterone and estrogens at the hypothalamic level and the negative action of progesterone on the pituitary by counteracting the estrogen sensitizing effect.

Acknowledgements—This study was supported in part by Ford Foundation Grant No. 67-470 and the WHO Reproduction Unit.

The GnRH preparation was kindly supplied by Ayerst Research Laboratories, Montreal, Canada. The reagents for the radioimmunossays of human FSH and LH were generously provided by the National Pituitary Agency (University of Maryland, School of Medicine) Endocrine Study Section and National Institute of Arthritis and Metabolic Diseases. The reagents for radioimmunossays of rat FSH and LH were made available to us through the generosity of Dr. A. Parlow for the Rat Pituitary Hormone Distribution Program NIH. The skilful performance of the *in vitro* studies with the rat pituitaries by Mr. A. Ben-Michael is greatly appreciated.

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