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SIMILARITIES AND DIFFERENCES IN PROGESTERONE AND ANDROGENS IN MODULATION OF LH, FSH AND PRL RELEASE: UNEXPECTED PROPERTIES OF FLUTAMIDE

DARRELL W. BRANN, CARLA D. PUTNAM and VIRENDRA B. MAHESH*
Department of Physiology and Endocrinology, Medical College of Georgia, Augusta, GA 30912-3000, U.S.A.

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Summary—The purpose of this study was to determine if the similarity of effect of progesterone and androgens on antagonism of estrogen-induced prolactin release also applied to the regulation of LH and FSH release. An additional objective was to examine the effect of the antiandrogen, flutamide, upon the ability of progesterone to induce gonadotropin secretion. Using the ovariectomized estrogen-primed immature rat, testosterone propionate suppressed LH and FSH secretion, whereas dihydrotestosterone only suppressed serum LH levels. In contrast, progesterone significantly elevated both serum LH and FSH levels. Thus, with respect to regulation of gonadotropin secretion, the effects of androgens and progesterone were dissimilar. In the estrogen-primed ovariectomized immature rat, flutamide was found to suppress LH, FSH and PRL secretion. Progesterone (0.8 mg/kg body wt) was incapable of overcoming this suppressive effect of flutamide. The effect of flutamide on gonadotropin secretion required estrogen priming. The effect of flutamide in suppressing LH, FSH and PRL release was not through suppression of an adrenal steroid as shown by adrenalectomy or the use of RU486. In the PMSG primed immature rat, flutamide had no effect on basal gonadotropin levels or ovulation. However, flutamide antagonized progesterone and triamcinolone acetonide-induced gonadotropin surges and blocked their ability to facilitate ovulation. These studies demonstrate that in the ovariectomized estrogen-primed immature rat flutamide has potent neuroendocrine regulatory ability leading to suppression of LH, FSH and PRL release. Flutamide also blocked progesterone and triamcinolone acetonide induced gonadotropin surges and ovulation in PMSG-primed immature female rats.

INTRODUCTION

Estradiol is recognized to be the primary trigger for the preovulatory gonadotropin surge [1, 2]. Recently, progesterone has also been demonstrated to be an important regulator of preovulatory gonadotropin secretion [3, 4]. Whereas the role of estradiol and progesterone in the regulation of gonadotropin secretion in the female is well documented, the role of androgens is not as well understood. In the female rat the increase in serum androgen levels occurs at the same time as the increase in estrogen levels, and it has been suggested that androgens may be important regulators of gonadotropin secretion, either directly or through aromatization to estrogens [5–7]. In a previous study, we demonstrated that dihydrotestosterone and progesterone were able to antagonize estrogen-induced prolactin release [8, 9]. Therefore, in addition to their direct effects, another mechanism of action of androgens in regulating gonadotropin secretion could be through modulation of the effect of estrogen. The purpose of this study was to use an ovariectomized estrogen-primed immature rat model to compare the effect of progesterone to that of an aromatizable androgen, testosterone, and a non-aromatizable androgen, dihydrotestosterone, on gonadotropin secretion. Furthermore, during our study of the effect of progesterone and dihydrotestosterone on estrogen-induced prolactin release, it was found that the antiandrogen, flutamide, blocked the action of progesterone in addition to blocking the effect of dihydrotestosterone [8]. Since flutamide is frequently used as an antiandrogen to study the role of androgens in female reproduction, the finding of antiprogestin-like activity warranted further investigation. Therefore an additional objective was to examine the effect of flutamide upon the ability of progesterone to induce gonadotropin secretion. These studies revealed some unexpected properties of flutamide.

*To whom correspondence should be addressed.

EXPERIMENTAL

Estrogen-primed ovariectomized rat model

Immature female Holtzman (virus-free) rats (Madison, Wis.) were obtained at 25 days of age and bilaterally ovariectomized on the next day under ether anesthesia. In studies in which adrenalectomized animals were utilized, adrenalectomy was performed at the same time as ovariectomy. The animals were maintained in air-conditioned rooms with a 14 h light: 10 h dark cycle (lights on at 0500 h; off at 1900 h EST) and were given water and rat chow *ad libitum*. Adrenalectomized rats received 0.9% NaCl drinking water. In the estrogen-primed experiments, all animals were injected subcutaneously with a 2 μg dose of estradiol (Steraloids) in 0.2 ml of 20% ethanol–saline vehicle at 1700 h at 27 and 28 days of age. On the third day (29 days of age), the animals received either vehicle (50% ethanol–saline) or steroids (s.c.) at 0900 h. This regimen of estrogen priming has been shown to induce progesterone receptors and progesterone sensitivity but by itself does not induce a gonadotropin surge [10]. In the experiments utilizing flutamide (4′-nitro-3′-fluoromethylisobutyranilide), the antiandrogen was administered intraperitoneally (i.p.) in propylene glycol at 0800 on day 29. In experiments utilizing RU486 (17β-hydroxy-11β-[4-dimethylaminophenyl]-17α-[prop-1-ynyl]-estra-4,9-diene-3-one), the antiprogestin (200 μg/rat) was adminsitered i.p. in 0.1 ml ethylene glycol 1 h prior to flutamide administration. All animals were killed by decapitation at 1500 h on day 29 and trunk blood was collected.

PMSG-primed immature rat model

Pregnant mare's serum gonadotropins (PMSG) (8 IU) was administered s.c. in saline vehicle to 28 day old immature female rats at 0800 h. 24 h later, the animals received progesterone (Steraloids) (2 mg/rat), triamcinolone acetonide (Sigma) (2 mg/rat) or vehicle (50% ethanol–saline) s.c. at 0800 h. Flutamide (5 mg/rat) was administered i.p. 1 h prior to progesterone or triamcinolone acetonide administration. For serum gonadotropin measurements the animals were sacrificed 10 h after steroid treatment (1800 h). Trunk blood was collected and allowed to clot for 12 h at 4°C, after which the blood was centrifuged at 2500 rpm for 30 min at 4°C and serum separated and stored at −20°C for subsequent radioimmunoassay of LH and FSH. In the experiments to determine facilitation of ovulation, groups of animals were sacrificed by decapitation 24 h after steroid treatment (0800 h) and the oviducts were removed, cleaned of fat, mounted and pressed on a slide and viewed under a microscope for the presence of ova.

Radioimmunoassay of LH, FSH and prolactin

The concentrations of LH, FSH and PRL in serum samples were analyzed by a double-antibody RIA method as described by Rao and Mahesh[4]. The purified hormones and standards and the first antibody for LH [NIAMDD-rLH-S-10 (rabbit)], FSH [NIAMDD-rFSH-S11 (rabbit)], and PRL [NIAMDD-r-PRL-S-9 (rabbit)] were obtained from NIDDK (National Hormone and Pituitary Program). The purified hormone was iodinated with ^{125}I (Amersham, Arlington Heights, Ill.) by the chloramine-T method [11]. The second antibody was purchased from Arnell Inc., Brooklyn, N.Y. A 25% binding was obtained at 1:46,825 and 1:25,000 dilutions for LH and FSH antisera, respectively; and at 1:2,500 dilution of the PRL antisera. The assay was linear at 4–128 ng/tube for LH, 32–512 ng/tube for FSH and 0.05–12.8 ng/tube for PRL. The intra- and interassay variabilities as determined by analysis of replicate serum pool samples were 9.6 and 12.4% for LH, 4.1 and 9% for FSH, and 7 and 11% for PRL. Hormone levels are expressed in terms of NIAMDD-RP-1 standard for LH and FSH and NIAMDD-RP-3 standard for PRL.

Progesterone radioimmunoassay

Progesterone was measured with a specific RIA procedure using D_3 antibody, which was well characterized and gave approximately 50% binding at 1:25,000 dilution [4]. The procedure involves ether extraction of serum. The dried ether extract was dissolved in phosphate buffer with gelatin and assayed for progesterone. The unbound hormone was removed by the dextran-coated charcoal absorption method, and the bound steroid was counted in a Beckman Scintillation spectrometer (Beckman Instruments, Palo Alto, Calif.). The assay was linear between 10–200 pg/tube. To avoid interassay variation all samples were saved and assayed together in one assay. The intraassay variation was 6%. Hormone levels are expressed as ng/ml serum.

Statistical analysis

The results given in the text are expressed as mean ± SEM. Significance of difference was assessed by one way analysis of variance and comparisons between treatment means were made by the Student–Newman–Keuls multirange test. $P < 0.05$ was considered significant.

RESULTS

Effects of progesterone and androgens upon LH and FSH in ovariectomized estrogen-primed immature rats

The administration of testosterone propionate at 0900 h on day 29 to ovariectomized immature rats primed with two consecutive daily injections of estradiol (2 μg/day) resulted in suppression of both serum LH and FSH levels (Fig. 1). Doses of 0.8, 1.6 and 10 mg/kg body wt of testosterone propionate significantly inhibited LH levels ($P < 0.01$), whereas the 0.4 mg/kg dose was ineffective. Doses of 1.6 and 10 mg/kg body wt of testosterone propionate were not more effective than the 0.8 mg/kg body wt dose

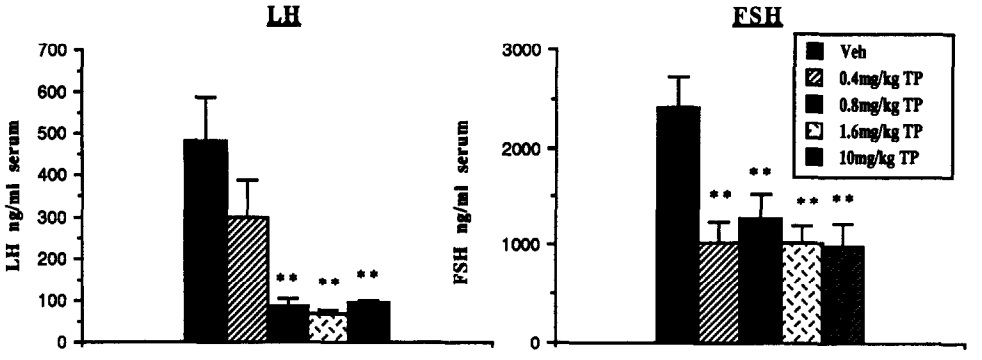

Fig. 1. Effect of testosterone propionate on LH and FSH secretion in estrogen-primed ovariectomized rats. Ovariectomized immature rats were administered estradiol (2 μg) on day 27 and day 28 (1700 h). On day 29 (0900 h) the animals received either vehicle (Veh), or testosterone propionate (TP). n = at least 6 animals per group, $^{**}P < 0.01$.

for LH suppression. Serum FSH levels were significantly suppressed by all four doses of testosterone propionate ($P < 0.01$) (Fig. 1) with no difference in the degree of suppression among them. The non-aromatizable androgen, dihydrotestosterone (DHT), significantly suppressed serum LH levels at doses of 0.4, 0.8, 1.6, 10 and 20 mg/kg body wt ($P < 0.05$–$P < 0.01$) (Fig. 2). There was no difference in the degree of suppression of serum LH with the different doses of DHT. DHT had no significant effect on serum FSH levels (Fig. 2). In contrast to the inhibitory effects of testosterone propionate and dihydrotestosterone, progesterone (0.8 mg/kg body wt) was found to significantly elevate both serum LH and FSH levels in estrogen-primed ovariectomized immature rats ($P < 0.01$) (Fig. 3).

Effects of flutamide on the progesterone-induced surges of LH, FSH and upon PRL serum levels in ovariectomized estrogen-primed rats

Progesterone administration, as described above, resulted in LH and FSH surges 6 h after administration in ovariectomized estrogen-primed immature rats (Fig. 3). Progesterone had no effect on serum PRL levels. The administration of the antiandrogen, flutamide, (5 mg/rat) 1 h prior to progesterone treatment completely blocked the progesterone-induced surges of LH and FSH ($P < 0.01$) (Fig. 3). PRL levels in the flutamide-progesterone treated group were also significantly reduced ($P < 0.01$) (Fig. 3). Moreover, flutamide, by itself, in the *absence* of progesterone administration, was found to significantly suppress LH, FSH and PRL serum levels in ovariectomized estrogen-primed rats ($P < 0.01$) (Fig. 3).

Effect of flutamide on the ability of progesterone and the glucocorticoid, triamcinolone acetonide, to facilitate gonadotropin secretion and ovulation in PMSG-primed immature rats

The administration of pregnant mare serum gonadotropin (PMSG) to immature female rats will cause a gonadotropin surge at 54–56 h after administration and ovulation 72 h after treatment [12]. However, progesterone administration 24 h after PMSG administration advances the gonadotropin surge and ovulation by 24 h [13]. We have recently demonstrated a similar effect by the glucocorticoid, triamcinolone acetonide [14]. As shown in Table 1, control animals, which received only PMSG, had an ovulation rate of 17%, an average of 1.2 ova/rat, and basal LH and FSH levels. Flutamide (5 mg/rat) had no significant effect on ovulation or gonadotropin

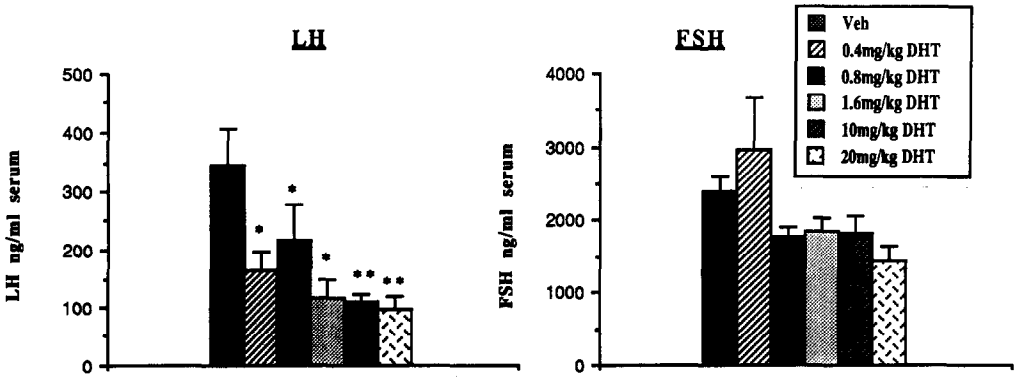

Fig. 2. Effect of dihydrotestosterone (DHT) on LH and FSH secretion in estrogen-primed ovariectomized rats. Ovariectomized immature rats were administered estradiol (2 μg) on day 27 and day 28 (1700 h). On day 29 (0900 h) the animals received either vehicle (Veh), or dihydrotestosterone (DHT). n = at least 6 animals per group, $^{*}P < 0.05$, $^{**}P < 0.01$.

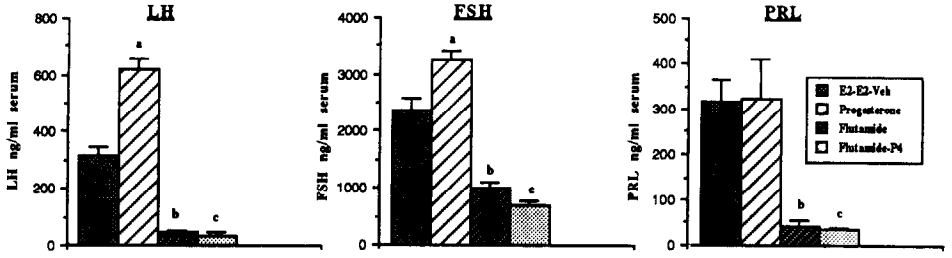

Fig. 3. Effect of flutamide on progesterone-induced gonadotropin secretion and prolactin release in estrogen-primed ovariectomized rats. The model is as described in Fig. 1. Flutamide's dose was 5 mg/rat, whereas progesterone's (P_4) dose was 0.8 mg/kg body wt. Flutamide was administered 1 h prior to progesterone. a = $P < 0.01$ vs controls; b = $P < 0.01$ vs controls; c = $P < 0.01$ vs progesterone-treated group.

levels. Both progesterone and triamcinolone acetonide increased % ovulation to approximately 95%, the number of ova to 6.9 ± 0.7 and 7.9 ± 0.9 respectively ($P < 0.01$), and both significantly increased serum LH and FSH levels over controls ($P < 0.01$). However, the administration of flutamide 1 h prior to progesterone treatment significantly decreased the % ovulation to 34% ($P < 0.01$), the number of ova/rat to 1.0 ± 0.3 ($P < 0.01$), and serum LH and FSH levels ($P < 0.01$). With respect to triamcinolone acetonide-induced ovulation and gonadotropin surges, flutamide pretreatment significantly decreased the % ovulation to 43% ($P < 0.01$), the number of ova/rat to 2.4 ± 1.4 ($P < 0.01$), and significantly inhibited triamcinolone acetonide-induced surges of LH and FSH ($P < 0.01$).

Effects of flutamide on serum LH, FSH and PRL in estrogen-primed vs non-estrogen-primed ovariectomized immature rats and dose dependency in estrogen-primed rats

As shown in Table 2, flutamide (5 mg/rat) had no effect on serum LH, FSH or PRL levels in *non*-estrogen-primed ovariectomized immature rats. In contrast, flutamide effectively suppressed all three hormones, using the 5 mg/rat dose, in estrogen-primed ovariectomized immature rats. A dose–response in estrogen-primed ovariectomized immature rats revealed that the lowest dose of flutamide (0.5 mg/rat) had no effect on serum LH or FSH levels, but significantly inhibited PRL serum levels ($P < 0.01$). The intermediate dose of flutamide, 2.0 mg/rat, effectively suppressed serum FSH and PRL levels in estrogen-primed ovariectomized rats ($P < 0.01$). Serum LH levels appeared to be lowered by the 2 mg/kg body wt dose of flutamide but the effect was not statistically significant. The 5 mg/kg body wt dose of flutamide effectively suppressed serum LH, FSH and PRL levels ($P < 0.05$, $P < 0.01$, $P < 0.01$, respectively) in estrogen-primed ovariectomized immature rats (Table 2).

Effect of flutamide on serum progesterone levels in ovariectomized rats and upon gonadotropin and prolactin release in adrenalectomized-ovariectomized rats

To determine if flutamide was suppressing LH, FSH and PRL serum levels via inhibiting adrenal function or some product secreted by the adrenal (i.e. adrenal corticoids or progestins), the following series of experiments were carried out.

In the first experiment, the effect of flutamide upon serum progesterone levels in ovariectomized rats was examined. Three groups of ovariectomized animals were utilized in this study: Group 1 received vehicle only; Group 2 received estradiol priming for two days; Group 3 received estradiol priming for two days and flutamide (5 mg/rat) on the third day. All animals were killed at 1500 h on the third day. As shown in Fig. 4, the estrogen-primed rats had significantly higher serum progesterone levels vs vehicle controls ($P < 0.05$). Flutamide treatment of the estrogen-primed rats resulted in a significant suppression of serum progesterone levels ($P < 0.01$ vs estrogen-primed controls), such that progesterone serum levels

Table 1. Effect of flutamide on the ability of progesterone (P_4) and triamcinolone acetonide (TA) to facilitate gonadotropin secretion and ovulation in PMSG-primed immature rat

Treatment group	Rats ovulating	Average ova/rat	Serum LH (ng/ml)	Serum FSH (ng/ml)
Control	17%	1.2 ± 0.5	92 ± 5	188 ± 25
Flutamide	16%	1.0 ± 0.4	105 ± 9	155 ± 23
P_4	94%	6.9 ± 0.7*	596 ± 76*	872 ± 94*
TA	95%	7.9 ± 0.9*	497 ± 95*	1101 ± 95*
Flutamide–P_4	34%**	1.0 ± 0.3**	229 ± 64**	242 ± 69**
Flutamide–TA	43%**	2.4 ± 1.4**	71 ± 8**	72 ± 22**

The doses used were: flutamide (5 mg/rat), progesterone (P_4) and triamcinolone acetonide (TA) (2 mg/rat), PMSG (8 IU/rat). Steroids were administered 24 h after PMSG. Flutamide was administered 23 h after PMSG. n = at least 6 animals per group. *Significant elevation vs controls ($P < 0.01$); **significant suppression as compared to P_4 or TA-treated groups ($P < 0.01$).

Table 2. Effect of flutamide on serum LH, FSH and PRL levels in estrogen-primed vs non-estrogen-primed ovariectomized immature rats

Treatment groups	LH (ng/ml)	FSH (ng/ml)	PRL (ng/ml)
Veh-Veh-Veh	192 ± 42	2442 ± 157	27 ± 3
Veh-Veh-Flutamide (5 mg)	177 ± 20	2273 ± 174	24 ± 3
E2-E2-Veh	220 ± 43	1985 ± 205	180 ± 40
E2-E2-Flutamide (0.5 mg)	280 ± 43	2056 ± 136	87 ± 12**
E2-E2-Flutamide (2.0 mg)	98 ± 27	1048 ± 136**	86 ± 16**
E2-E2-Flutamide (5 mg)	44 ± 8*	1309 ± 93**	34 ± 6**

The model is the same as described in Figs 1 and 3. Non-estrogen-primed animals received vehicle instead of estradiol. n = at least 6 animals per group. *$P < 0.05$; **$P < 0.01$.

in these estrogen-primed rats were no longer different from the vehicle controls.

To conclusively rule out that flutamide's inhibition of LH, FSH and PRL serum levels was due to suppression of adrenal function or through inhibition of some adrenal product, rats were ovariectomized and adrenalectomized, primed with estradiol, and the ability of flutamide to inhibit LH, FSH and PRL levels in these animals was assessed.

As shown in Fig. 5, the removal of the adrenal did not alter flutamide's ability to inhibit LH, FSH or PRL serum levels ($P < 0.01$).

Effect of the antiprogestin, RU486, on flutamide inhibition of LH, FSH and PRL release

The requirement of estrogen priming for flutamide's effect could possibly indicate progesterone receptor mediation since progestin receptor concentrations are strongly dependent on estrogen for induction. Even though in the preceding experiment, the role of flutamide in inhibiting adrenal steroids was ruled out by adrenalectomy, further confirmation was sought by using the progesterone and corticosteroid antagonist RU486. The administration of RU486 did not bring about any change in serum LH, FSH and PRL levels as compared to vehicle-treated controls, indicating that adrenal steroids that could be antagonized by RU486 did not play any role in the basal levels of LH, FSH and PRL in estrogen-primed vehicle treated immature ovariectomized rats (Fig. 6). RU486 (200 μg/rat) administered 1 h prior to flutamide administration in the estrogen-primed ovariectomized rat was also ineffective in blocking flutamide's action of suppressing LH, FSH and PRL serum levels (Fig. 6).

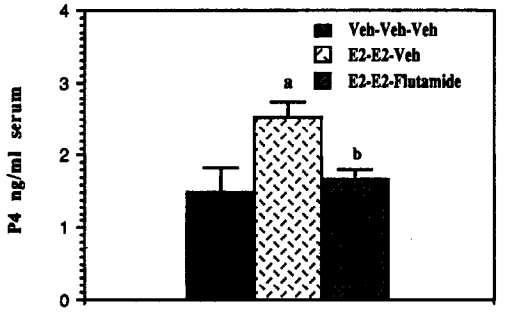

Fig. 4. Effect of flutamide on progesterone serum levels in ovariectomized immature rats. The model is the same as described in Figs 1 and 3. Non-estrogen-primed ovariectomized rats (Veh-Veh-Veh) which received only vehicle are included for comparison to estrogen treated animals. Flutamide was used at a dose of 5 mg/rat. n = at least 6 animals per group. a = $P < 0.05$ vs Veh only (Veh-Veh-Veh) group; b = $P < 0.05$ vs E_2-E_2-Veh group.

DISCUSSION

Previous work from our laboratory has demonstrated that progesterone causes a rapid depletion of occupied nuclear estradiol receptors in the anterior pituitary [15, 16]. During this period of progesterone effect on pituitary estradiol receptors, the ability of estradiol to induce pituitary progesterone receptors and prolactin release was found to be impaired [9, 17]. Subsequent studies have demonstrated that corticosteroids and androgens behave similarly to progesterone in antagonizing estrogen-induced prolactin release [8, 10]. Hence, the purpose of this study was to determine if the similarity of effect of progesterone and androgens applied only to antagonism of estrogen-induced prolactin secretion or also to the regulation of LH and FSH release. Such a study was made possible by the use of the estrogen-primed ovariectomized immature rat model characterized previously, in which estrogen priming is sufficient to induce progesterone sensitivity [17] but not estrogen-induced gonadotropin surges [10].

Using this model, the aromatizable androgen, testosterone propionate, was found to significantly inhibit both serum LH and FSH levels (Fig. 1). Dihydrotestosterone suppressed serum LH levels but had no significant effect on FSH levels (Fig. 2). Dihydrotestosterone was more potent in suppressing serum LH levels since the 0.4 mg/kg body wt of dihydrotestosterone was effective while the same dose of testosterone propionate was ineffective (Fig. 1 vs Fig. 2). Other investigators have also reported dihydrotestosterone to be more potent than testosterone in inhibiting LH secretion [7, 18]. The lack of a significant effect on serum FSH levels by dihydrotestosterone, whereas testosterone propionate significantly suppresses serum FSH levels, may suggest that FSH suppression by androgens is through aromatization to estrogens. In support of this, estrogens have been shown to be potent inhibitors of FSH secretion in many situations [1]. In contrast to the inhibitory effects of testosterone propionate and dihydrotestosterone, progesterone significantly

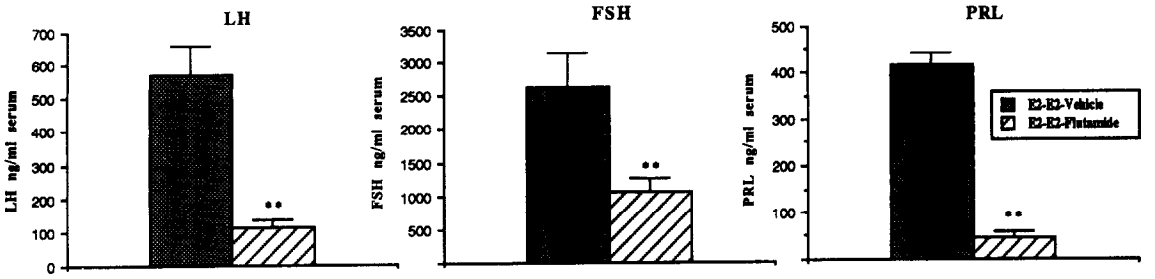

Fig. 5. Effect of flutamide upon serum LH, FSH and PRL levels in estrogen-primed ovariectomized and adrenalectomized immature rats. The model is the same as described in Figs 1 and 2 except adrenalectomy was also perfomed at the time of ovariectomy. The dose of flutamide was 5 mg/rat. n = at least 6 animals per group, $^{**}P < 0.01$.

elevated both serum LH and FSH levels (Fig. 3). Thus, using our model, the effects of progesterone and androgens on gonadotropin secretion were found to be dissimilar. This is in agreement with the majority of reports in the literature [3, 6, 7, 18, 19].

In a previous study we reported that the antiandrogen, flutamide, blocked progesterone's effect of inhibiting estrogen-induced prolactin release [8]. Flutamide's active metabolite, hydroxyflutamide, has also been reported to block the proestrous surge of LH and ovulation in PMSG-primed immature rats [20], an effect which could be overcome by progesterone but not testosterone [21]. Therefore a second objective of this study was to examine flutamide's effect on progesterone-induced gonadotropin secretion.

In the estrogen-primed ovariectomized immature rat, flutamide (5 mg/rat) was found to suppress LH, FSH and PRL secretion *directly* in the absence of progesterone administration (Fig. 3). Progesterone (0.8 mg/kg body wt) was incapable of overcoming the suppression of serum LH, FSH and PRL levels by flutamide (Fig. 3). Flutamide significantly suppressed progesterone secretion in estrogen-primed ovariectomized rats (Fig. 4). However, the effect of flutamide in suppressing gonadotropin and prolactin release was not through suppression of adrenal secretion or inhibition of adrenal products such as progesterone or corticoids, as evidenced by its activity in adrenalectomized-ovariectomized rats and in RU486-treated rats (Figs 5 and 6). These findings suggest that the inhibitory effect of flutamide is mediated directly at the hypothalamic–pituitary level without adrenal or ovarian involvement. The ability of flutamide to suppress the release of LH, FSH and PRL release is not due to a "toxic" effect of flutamide. If this were the case, flutamide should also have suppressed LH, FSH and PRL release in the *non*-estrogen-primed ovariectomized rat; it did not (Table 2). The dose of flutamide used in our studies, 5 mg/rat, is a common dose used in a majority of the reports in the literature for antagonizing androgen action [20–22]. A dose–response for flutamide revealed that PRL secretion was effectively suppressed by a 10-fold lower dose of flutamide (0.5 mg/rat); serum FSH levels were suppressed by a 2.5-fold lower dose of flutamide (2 mg/rat), whereas serum LH levels were only significantly suppressed by the 5 mg/rat dose of flutamide (Table 2). The suppressive effect of flutamide on gonadotropin secretion appears to require elevated levels of gonadotropins. When gonadotropin levels are basal as found in the PMSG-primed rat, flutamide was found not to reduce serum LH and FSH levels even though estrogens are present as evidenced by sensitivity to progesterone (Table 1). However, flutamide did significantly antagonize progesterone and triamcinolone acetonide-induced gonadotropin surges and blocked their ability to facilitate ovulation in the PMSG-primed rat. It is unclear whether this is an antiprogestational effect of flutamide or a direct effect inhibiting the systems responsible for the release of gonadotropins. On the other hand, flutamide can suppress gonadotropin secretion in the absence of progesterone when

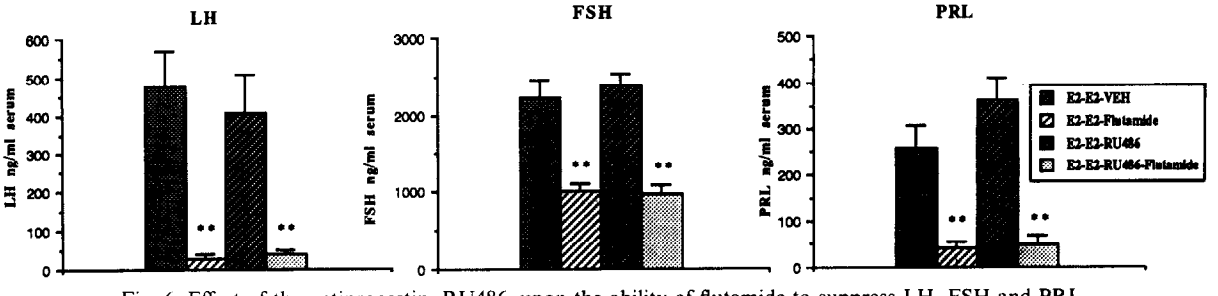

Fig. 6. Effect of the antiprogestin, RU486, upon the ability of flutamide to suppress LH, FSH and PRL release in estrogen-primed ovariectomized immature rats. The model is the same as described in Figs 1 and 3. RU486 (200 μg/rat) was injected 1 h prior to flutamide (5 mg/rat), n = at least 6 animals per group $^{**}P < 0.01$.

gonadotropin levels are high. In our estrogen-primed ovariectomized rat model, serum gonadotropins in the non-estrogen primed rats are elevated in the morning hours as compared to estrogen-primed rats [10]. By the time of sacrifice at 1500 h, the levels in both groups are similarly elevated because the last estrogen injection was given 22 h earlier (Table 2). In estrogen primed animals, flutamide reduced serum LH and FSH levels while it was unable to do so in the absence of estrogen priming (Table 2). Thus a gonadotropin suppressive effect of flutamide is demonstrated in the absence of progesterone and in the presence of estrogen priming and high gonadotrpin levels. This indicates a direct effect on gonadotropin secretion under these conditions.

The mechanism of action of flutamide in suppressing LH, FSH and PRL release remain unclear. The requirement of estrogen priming could possibly suggest progesterone receptor involvement. However, progesterone receptor mediation of flutamide's effects in our study seems unlikely as evidenced by the failure of the potent progesterone receptor antagonist, RU486, to block flutamide's effects (Fig. 6). Additionally, Chandrasekhar and Armstrong recently reported that hydroxyflutamide does not bind to the progesterone receptor in the rat uterus [23].

An alternative explanation for the requirement of estrogen priming for flutamide's effect is that flutamide is acting through pituitary androgen receptors, which have recently been reported to be upregulated by estrogen [24]. It is intriguing that dihydrotestosterone, and especially testosterone propionate, exhibited suppressive effects on serum LH and FSH levels similar to flutamide (cf. Figs 1, 2 and 3). Also intriguing is the previous report that dihydrotestosterone and 5α-androstane-3α,17β-diol are capable of suppressing progesterone-induced LH and FSH surges in the estrogen-primed immature female rat [25]. These similarities between the effects of androgens and flutamide would seem to suggest androgen receptor mediation of flutamide's effects in our study. Further studies are required to answer this question.

In summary, this study demonstrates that in the ovariectomized estrogen-primed immature rat flutamide has potent neuroendocrine regulatory ability leading to suppression of LH, FSH and PRL release. In the PMSG-primed intact immature rat flutamide blocked progesterone and triamcinolone acetonide induced gonadotropin surges and ovulation. In light of these findings, caution may be advisable in the use and interpretation of flutamide results in the female rat.

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