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STIMULATORY AND INHIBITORY EFFECTS OF PROGESTERONE ON FSH SECRETION BY THE ANTERIOR PITUITARY

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Summary—The purpose of this study was to investigate whether progesterone exerted progesterone receptor mediated direct effects on the anterior pituitary in the secretion of FSH and whether such effects were mediated through the 5α-reduction of progesterone. Treatment of anterior pituitary dispersed cells for 48 h with 0.5 nM estradiol reduced the ED_{50} for gonadotropin releasing hormone (GnRH)-stimulated FSH release from 0.58 to 0.36 ng/ml and the ED_{50} for GnRH-induced LH release from 0.54 to 0.19 ng/ml. When dispersed pituitary cells were treated with 0.5 nM estradiol and exposed to various doses of progesterone for 1 to 6 h, the most consistent rise in basal and GnRH-stimulated FSH release was observed with the 50 nM dose of progesterone with a 3-h exposure period. All three doses of progesterone elevated basal LH and GnRH-stimulated LH was increased by the 50 and 100 nM doses of progesterone during the 3-h period of treatment. Using the 50 nM dose of progesterone, basal and GnRH-stimulated LH was increased after 2, 3 and 6 h of progesterone treatment. When the period of exposure of progesterone was extended to 12, 36 or 48 h, there was a significant inhibition of GnRH-stimulated FSH release. GnRH-stimulated LH release was inhibited at 36 and 48 but not 12 h after progesterone treatment. These studies showed that the effect of progesterone administered for periods of 1 to 6 h enhanced the secretion of LH and FSH whereas progesterone administered for periods beyond 12 h inhibited FSH and LH release by dispersed pituitary cells in culture. These results are similar to those observed *in vivo* after progesterone treatment. Furthermore estrogen priming of the dispersed pituitary cells was necessary to observe the effects of progesterone. The progesterone antagonist RU486 prevented the progesterone-induced rise in GnRH-stimulated FSH release. Furthermore the 5α-reductase inhibitor *N,N*-diethyl-4-methyl-3-oxo-4-aza-5α-androstane-17β-carboxamide also prevented the progesterone-induced rise in GnRH-stimulated FSH release in estrogen-treated dispersed pituitary cells. These results indicate that the anterior pituitary is a major site of action of progesterone in the release of FSH and that 5α-reduction of progesterone plays an important role in FSH release.

INTRODUCTION

The release of FSH and LH is regulated by a single hypothalamic-releasing factor, gonadotropin releasing hormone (GnRH) [1]. However, there are a number of physiological and experimentally-induced situations where the secretion of the gonadotropins, FSH and LH is divergent. For instance during the rat estrous cycle, the elevation of LH levels during the preovulatory surge is of a relatively short duration but serum FSH remains elevated during the night and on estrus [2]. Furthermore, in the adult rat, ovariectomy results in elevated serum levels of FSH shortly after castration, whereas with serum LH levels become elevated only after a delayed period depending upon the stage of the cycle at which ovariectomy was carried out [3]. Estradiol administration to ovariectomized rats also causes a difference in the suppression of elevated serum gonadotropins, with LH declining to baseline more rapidly than FSH [4]. The administration of progesterone to estrogen-primed ovariectomized rats has been shown to bring about both FSH and LH release with the FSH release being prolonged similar to events during proestrus and estrus in the rat [5, 6]. Similarly, in normal women, estrogen benzoate given alone induced an increase in LH secretion, whereas, when progesterone was administered during the last three days of estradiol benzoate treatment, the surge of LH was

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enhanced and was accompanied by a rise in serum FSH levels [7]. Progesterone has also been shown to stimulate FSH secretion in the hamster [8] and in the rhesus monkey [9]. Recent findings in our laboratory have demonstrated that a metabolite of progesterone, 5α-dihydroprogesterone (5α-DHP), caused the selective secretion of FSH in immature ovariectomized estrogen-primed rats and immature pregnant mare's serum gonadotropin (PMSG) treated rats [10, 11].

Progesterone receptors have been found in hypothalamic tissue [12–16] and on gonadotropes in the anterior pituitary gland [16]. Thus, progesterone may regulate gonadotropin secretion by its action at the hypothalamus, the anterior pituitary, or at both sites. Progesterone has been found to bring about the rapid release of hypothalamic GnRH [17, 18]. Dispersed anterior pituitary cell cultures have been used extensively to determine whether progesterone exerts a direct effect on the anterior pituitary in facilitating LH and FSH secretion. Work by a number of investigators using anterior pituitary cell cultures has shown that progesterone exerts a direct modulatory effect on LH secretion [19–23]. The regulation of the secretion of FSH, however, is less clearly understood. This may, perhaps, be due to the tighter correlation of LH secretion with GnRH stimulation as compared to FSH, resulting in variable responses. In some studies in the rat, progesterone appeared to have facilitory effects on FSH secretion [19, 21, 22].

The purpose of this study was to investigate (a) whether progesterone had direct effects on the pituitary in the secretion of FSH in response to GnRH, (b) whether the direct effect of progesterone on FSH secretion from anterior pituitary cells was manifested through its interaction with the progesterone receptor, and (c) whether 5α-reduction of progesterone was necessary for its action in the release of FSH. Parallel studies were carried out with LH release in our pituitary cell cultures to ensure that the results obtained were comparable to those reported in the literature to validate the *in vitro* cell culture conditions.

MATERIALS AND METHODS

Animals

Adult female virgin virus-free Holtzman rats were obtained at 53 days of age and maintained in temperature-controlled rooms with 14 h of light and 10 h of darkness (lights on at 0500 h and off at 0900 h EST) and given water and rat chow *ad libitum*. The rats were bilaterally ovariectomized under ether anesthesia. After 7 days of ovariectomy, the animals were killed by decapitation. The pituitary glands were removed and after dissecting out the posterior pituitaries, the anterior pituitary glands were placed in sterile Dulbecco's Modified Eagle's Medium (DMEM), (Gibco 430-3000 EB), without phenol red containing 5% charcoal-stripped fetal calf serum (Sigma F3010) and 5% charcoal-stripped horse serum (Sigma H7889), penicillin–streptomycin 10,000 U/ml/10,000 μg/ml (Gibco 600-5145 AE) and 1% nonessential amino acids (Gibco 320-1140).

Establishment of a cell culture system

Adenohypophyseal cell cultures were established with modifications of the methods previously described by O'Conner *et al.* [24]. Briefly, anterior pituitary tissue was first mechanically dispersed by mincing into 1 mm cubes and then added to 0.25% crude trypsin solution (Gibco 610-5050 AG) at 0.2 ml/pituitary and allowed to stir with a mini stir bar on a stir plate. Enzymatic dispersal was carried out for 1 h at 37°C in a humidified atmosphere of 95% air and 5% CO_2. The enzymatic dispersal was terminated by addition of soybean trypsin inhibitor (Sigma T-9128; 1 mg/ml/pituitary) to the digest followed by filtration through sterile gauze. The filtrate was then centrifuged at 500 *g* for 10 min at room temperature and the supernatant was discarded and the resulting pellet was resuspended in DMEM complete with supplemental sera, antibiotics, and nonessential amino acids. An equivalent of one half of a dispersed anterior pituitary was plated in each 35 × 10 mm culture dish in a volume of 0.2 ml and the volume was then brought up to a 1.0 ml with complete medium. Approx. 2×10^6 cells per dish were plated with a viability routinely obtained at 90% as determined by erythrosin B exclusion dye. The medium was removed and replaced every 24 h. Cells were allowed to preincubate for 48 h before 48-h steroid treatments were initiated. Cells were then stimulated with GnRH for 3 h at which time the hormone released into the medium was collected and quantified by radioimmunoassay (RIA).

RIA

Medium content and cellular content of LH and FSH were quantified by a modified version

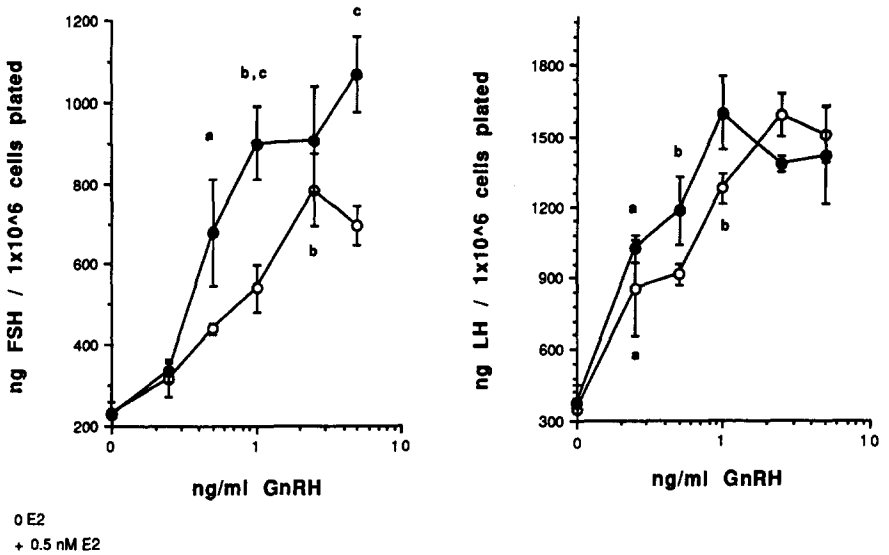

Fig. 1. GnRH dose–response curve in the presence or absence of 0.5 nM estradiol (E_2). Cells were allowed to preincubate for 48 h with one-half medium change at 24 h. They were then incubated for 48 h with or without 0.5 nM estradiol. Medium was changed with fresh estradiol in estrogen-treated cells and at the termination of the 48 h of estrogen priming after 24 h of estrogen exposure. The cells were then stimulated with GnRH (0.25, 0.5, 1.0, 2.5 and 5.0 ng/ml) for 3 h at which time medium was collected and assayed for FSH and LH. **a** $P < 0.05$ first significant increase above nonGnRH-stimulated controls, **b** $P < 0.05$ maximum level of hormone release, and **c** $P < 0.05$ as compared to nonestrogen treated controls.

of the double antibody RIA of Hunter and Greenwood [25]. The purified hormones, standards and antibodies were obtained from NIDDK, The Pituitary Hormone Distribution Program, NIH. Purified hormones were iodinated with Na ^{125}I by the chloramine T method of Bolton [26]. The first antibodies used for LH and FSH were anti-rLH-S-10 and anti-rFSH-S-11, AFP-CO972881 (NIDDK), respectively. Samples were incubated with the first antibody for 24 h, followed by the addition of the second antibody (goat antirabbit gamma globulin, Arnell Inc., NY, lot No. G103252) for 18 h. Samples were centrifuged at 3000 rpm (860 *g*) for 45 min at 4°C. The supernatants were aspirated and pellets were counted in a Beckman gamma counter (Beckman g-8000 counting system, Palo Alto, CA). Approx. 25% binding was routinely obtained at 1:56,250 and 1:18,750 dilutions for LH and FSH antisera, respectively. Standard curves were performed in triplicate. The intra- and interassay variabilities as determined by analysis of replicate serum pools samples were 8.7 and 12.2% for LH and 10.1 and 14.8% for FSH. Standards were rLH-RP-3 and rFSH-RP-2. Unknowns were assayed in duplicate and calculated to a weighted raw data linear regression. Hormone levels were expressed in terms of NIAMDD-RP-1 standard for LH and FSH.

Statistical analysis

Data was expressed as ng of hormone per 1×10^6 cells plated. The results are expressed as mean ± SEM. Analysis of variance (ANOVA) was performed to determine if differences between means exist and significance at the $P < 0.05$ level was determined by Fisher's protected least significant difference (PLSD).

RESULTS

Establishment of a dose–response curve to GnRH

Conditions had to be established to determine the responsiveness of the anterior pituitary cell cultures to GnRH. Since estradiol is known to enhance the sensitivity of pituitary responsiveness to GnRH, and since a dose of 0.5 nM estradiol has been previously shown to enhance pituitary sensitivity *in vitro*, a dose–response curve to GnRH was established using the doses of 0.25, 0.5, 1.0, 2.5 and 5.0 ng/ml of GnRH added to dispersed pituitary cells for 3 h that had been previously incubated for 48 h in the presence or absence of 0.5 nM estradiol. For FSH, the first significant increase in secretion occurred at 0.5 ng/ml of GnRH and the maximal effect was reached at 2.5 ng/ml of GnRH by cells in the absence of estradiol priming. In cells primed with 0.5 nM estradiol, the first

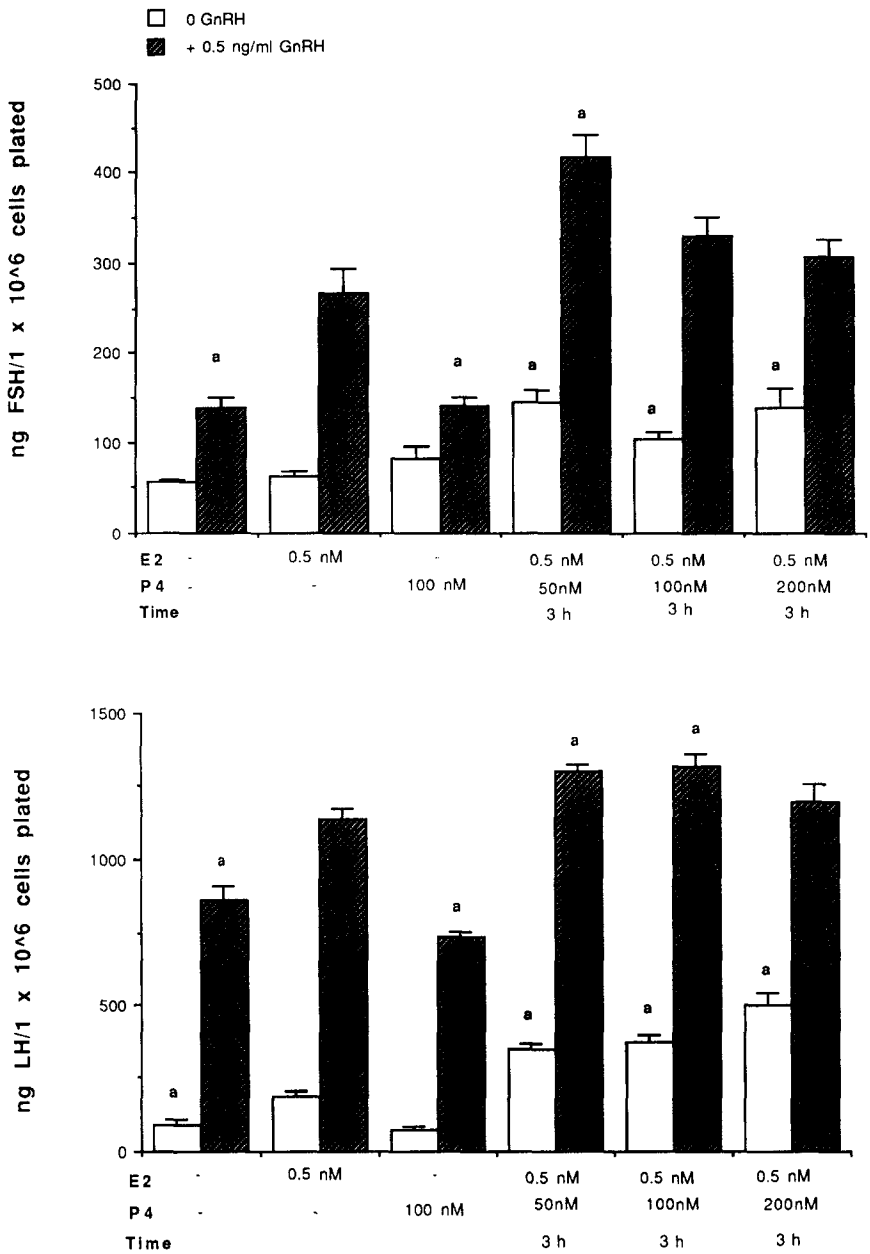

Fig. 2. Effect of 3 h of progesterone (P_4) on FSH and LH release in estrogen-primed dispersed anterior pituitary cells. Following 48 h of estrogen priming (0.5 nM E_2), cells were exposed to 3 h of progesterone (50, 100 or 200 nM) and then washed twice with plain medium before 3 h of stimulation with GnRH (0.5 ng/ml). Cells exposed to estradiol vehicle (0.00001% ETOH) for 48 h followed by 3 h of progesterone vehicle (0.01% ETOH) or progesterone (100 nM) without estradiol priming served as controls. **a** $P < 0.5$ as compared to estrogen-treated controls.

significant rise in FSH was also induced by 0.5 ng/ml of GnRH but the maximum increase in FSH was induced by 1.0 ng/ml GnRH. The ED_{50} for GnRH-stimulated FSH secretion was reduced in the presence of 0.5 nM estradiol priming from 0.58 to 0.36 ng/ml (Fig. 1). The first significant increase in LH secretion occurred at 0.25 ng/ml of GnRH and the maximal effect was reached at 1.0 ng/ml of GnRH in the absence of estradiol. In estrogen-primed cells, the first significant rise in LH was also observed after exposure to 0.25 ng/ml GnRH but the maximum secretion of LH required only 0.75 ng/ml of GnRH. The ED_{50} for GnRH-induced LH release was determined to decrease in the presence of estradiol from 0.54 to 0.19 ng/ml (Fig. 1). The dose of 0.5 ng/ml GnRH was chosen for use in further studies because it stimulated the secretion of both gonadotropins that were intermediate to

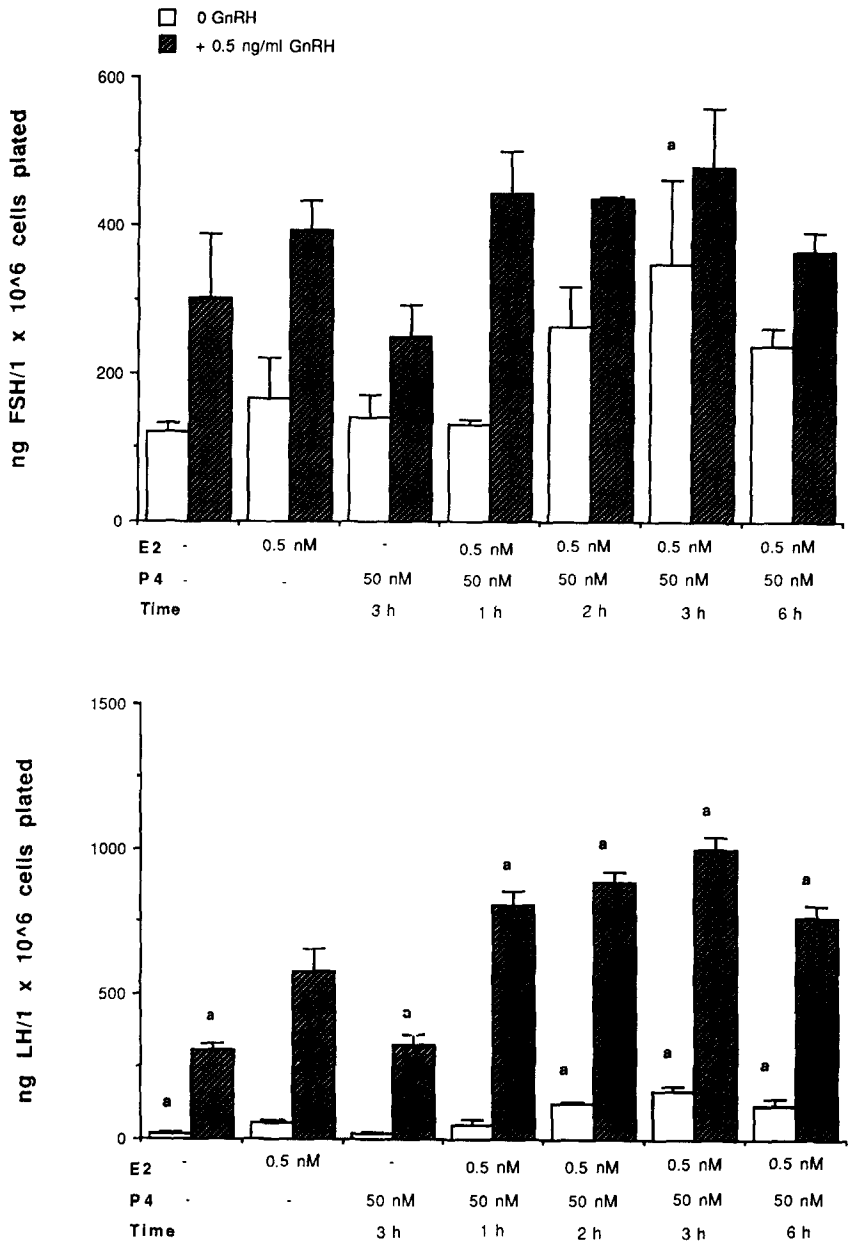

Fig. 3. The effect of 1 to 6 h of progesterone (P_4) exposure on FSH and LH secretion in dispersed anterior pituitary cells. Following 48 h of estrogen priming (0.5 nM E_2), cells were exposed to 1, 2, 3 or 6 h of progesterone (50 nM) and then washed twice with plain medium before 3 h of stimulation by GnRH (0.5 ng/ml). Cells exposed to estradiol vehicle (0.00001% ETOH) for 48 h followed by 3 h of progesterone vehicle (0.01% ETOH) or progesterone (50 nM) without estradiol priming served as controls. a $P < 0.05$ as compared to estrogen-treated controls.

the first significant rise and to the maximum secretion.

Acute effects of progesterone on FSH and LH release in estrogen-treated dispersed pituitary cells

Three doses of progesterone were first tested using 50, 100, and 200 nM for 3 h following 48 h of estrogen priming (Fig. 2). Progesterone (100 nM), in the absence of estrogen priming (0.5 nM), was not effective in modulating FSH release. Following 48 h of estrogen priming, all three doses of progesterone enhanced basal FSH above the estrogen-treated controls ($P < 0.05$). GnRH-stimulated FSH secretion was significantly enhanced by the 50 nM dose of progesterone above the estrogen-treated control ($P < 0.05$). However, the 100 and 200 nM doses of progesterone did not alter GnRH-stimulated FSH secretion compared to the estrogen-treated

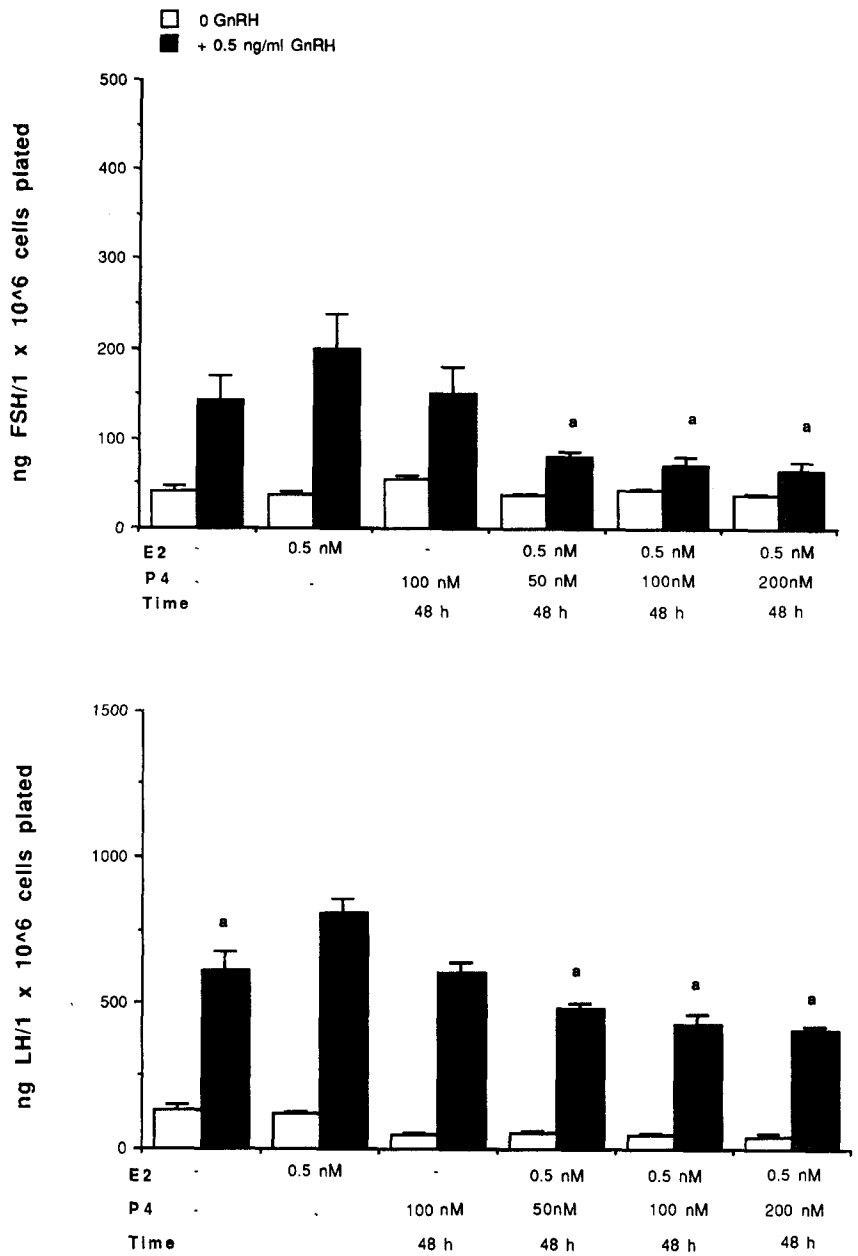

Fig. 4. The effect of 48 h of progesterone (P_4) exposure on FSH and LH release in dispersed anterior pituitary cells. Cells were concomitantly exposed with 48 h of estradiol (0.5 nM E_2), and 48 h of progesterone (50, 100, or 200 nM) and then washed twice with plain medium before 3 h of stimulation by GnRH (0.5 ng/ml). Cells exposed to vehicle (0.01% ETOH) or progesterone (100 nM) without estradiol priming for 48 h served as controls. a $P < 0.05$ as compared to estrogen-treated controls.

control. Progesterone (100 nM) in the absence of estrogen priming, was not effective in modulating LH release. However, following 48 h of estrogen priming, all three doses of progesterone elevated basal LH levels above the estrogen-treated control ($P < 0.05$). The 50 and 100 nM doses of progesterone significantly enhanced GnRH-stimulated LH secretion ($P < 0.05$), whereas, the 200 nM dose was ineffective.

Since the 50 nM dose of progesterone was effective in enhancing the basal and GnRH responses of both FSH and LH levels, it was chosen for use in further experiments. The time required for progesterone to exert its modulatory effect on FSH and LH secretion was studied next. Anterior pituitary cells were exposed to 50 nM of progesterone for 1, 2, 3, and 6 h subsequent to 48 h of estrogen priming (0.5 nM) (Fig. 3). Cells were exposed to progesterone (50 nM) for 3 h in the absence of estrogen priming to serve as a control. Progesterone, in the absence of estrogen priming, had no effect

on either basal or GnRH-stimulated FSH release. 3 h of progesterone treatment caused an elevation of basal FSH release over the estrogen-treated control ($P < 0.05$), whereas, exposure to 1, 2 or 6 h did not significantly elevate basal FSH even though the mean levels were higher than estrogen-treated controls. GnRH-stimulated FSH secretion was not significantly enhanced at any of the four times examined. Anterior pituitary cells exposed to progesterone (50 nM) in the absence of estrogen priming did not significantly alter either basal or GnRH-stimulated LH levels. Exposure of cells to 2, 3 and 6 h of progesterone significantly elevated basal LH levels above estrogen-treated controls ($P < 0.05$) with no effect on basal LH levels at 1 h. A stimulatory effect of progesterone on GnRH-stimulated LH release was observed as early as 1 h after exposure ($P < 0.05$). Additionally, exposure to progesterone for 2, 3 and 6 h was also effective in enhancing GnRH-stimulated LH release ($P < 0.05$).

Effect of 48-h progesterone treatment on FSH and LH release in estrogen-treated dispersed pituitary cells

To determine what effect long-term exposure of cells to progesterone would have on estrogen-induced GnRH sensitization, anterior pituitary cells were exposed to progesterone concomitantly with estradiol (0.5 nM) for 48 h (Fig. 4). Cells were then exposed to GnRH for 3 h. Exposure to 48 h of progesterone (100 nM) in the absence of estrogen priming, did not have any effect on basal or GnRH-stimulated FSH. Concomitant with 48 h of estrogen priming, progesterone, at all three doses tested above (50, 100 and 200 nM) did not alter basal FSH levels yet significantly inhibited GnRH-stimulated FSH secretion as compared to estrogen-treated controls ($P < 0.05$). 48 h of progesterone exposure (100 nM) in the absence of estradiol priming did not alter either basal or GnRH-stimulated LH release. Concomitant with 48 h of estrogen priming, progesterone did not affect basal LH levels, at any of the three doses tested, yet significantly inhibited GnRH-stimulated LH release compared to estrogen-treated controls with all three doses tested ($P < 0.05$).

The time required for progesterone to exert its inhibitory effect on FSH and LH secretion was next examined using 50 nM progesterone (Fig. 5). Progesterone was added to the medium for either the full 48 h, for the last 36 h, or for the last 12 h of the 48 h of estradiol incubation. Progesterone had no statistically significant effect on basal FSH levels compared to estrogen-treated controls. However, exposure of cells to 48, 36, or 12 h of progesterone significantly inhibited GnRH-stimulated FSH secretion compared to estrogen-treated controls. Basal LH levels were not affected by progesterone at any time of exposure tested. However, exposure of cells to 48 or 36 h of progesterone concomitant with estradiol priming caused a significant inhibition of GnRH-stimulated LH release. Exposure of cells to progesterone for the last 12 h of 48-h estrogen priming did not affect GnRH-stimulated LH release.

Effect of the antiprogestin RU486 on progesterone-induced gonadotropin release

To determine if the action of progesterone on FSH secretion in dispersed anterior pituitary cells was mediated through the progesterone receptor, the use of the progesterone receptor antagonist, RU486 (17β-hydroxy-11β-[4-dimethylaminophenyl]-17α-[prop-1-ynyl]-estra-4,9-diene-3-one), was made. To first test if RU486 itself would affect gonadotropin secretion, cells were exposed to 2 h of RU486 (100 nM), following 48 h of estrogen priming (0.25 nM). RU486 did not affect either basal or GnRH-stimulated FSH secretion. 100 nM RU486 completely antagonized the stimulatory effect of 50 nM progesterone on GnRH-stimulated (0.25 ng/ml) FSH secretion ($P < 0.05$), (Fig. 6). RU486 itself, had a stimulatory effect on basal LH levels. When co-incubated with progesterone for 2 h, RU486 inhibited the elevation in basal LH release induced by progesterone, with no effect on GnRH-stimulated LH release (Table 1). RU486 by itself, in the absence of estrogen priming did not alter basal (control: 266.8 ± 42.1 vs RU486: 325.1 ± 87.7 ng/10^6 cells) or GnRH-stimulated (control: 545.7 ± 119.4 vs RU486: 689.9 ± 68.2 ng/10^6 cells) LH or FSH (basal control: 375.0 ± 24 vs RU486: 244.3 ± 33.4 ng/10^6 cells; GnRH-stimulated control: 385.3 ± 38.4 vs RU486: 287.7 ± 4.0 ng/10^6 cells) release.

Effect of the 5α-reductase inhibitor 4-MA on progesterone-stimulated FSH secretion

Since in earlier studies, 5α-dihydroprogesterone administration brought about a selective release of FSH *in vivo*, it was of interest to determine if 5α-reduction of progesterone was

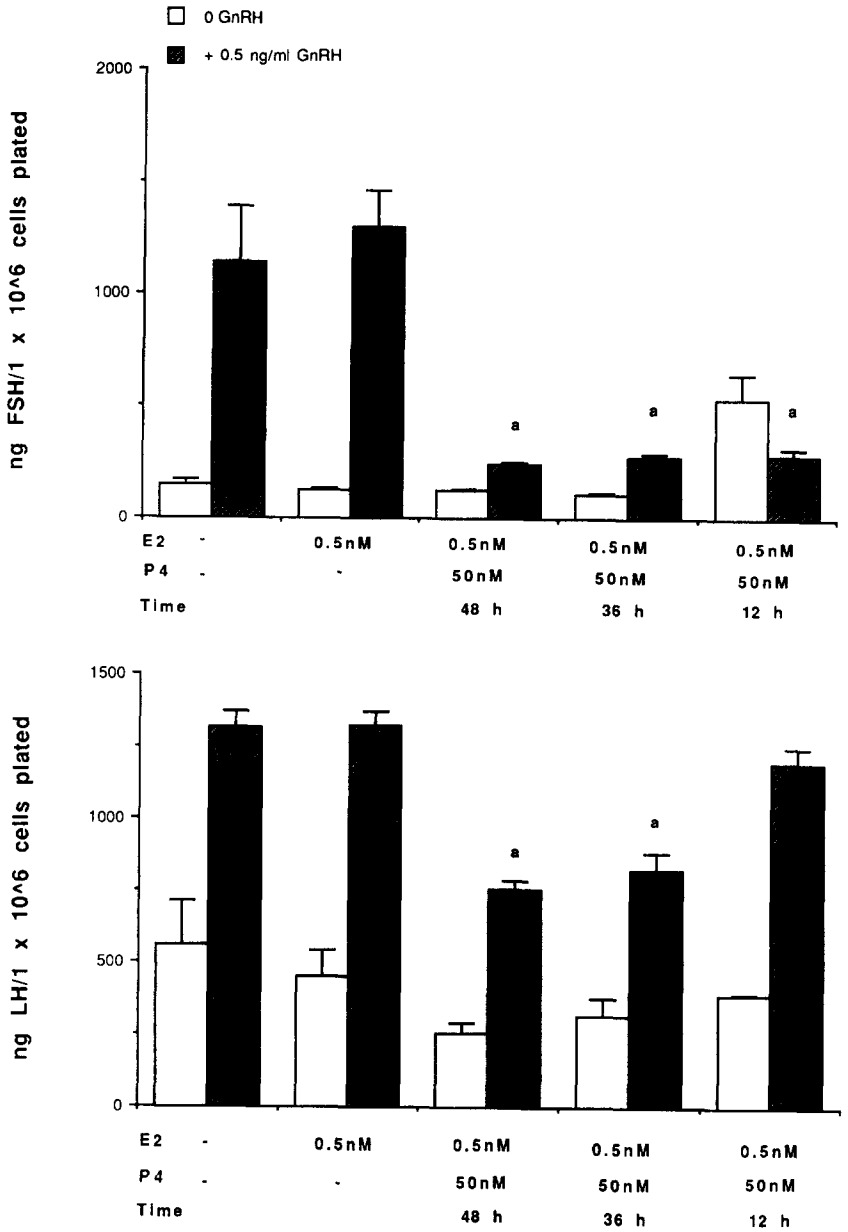

Fig. 5. Effect of 12–48 h of progesterone (P_4) exposure on FSH and LH release in dispersed anterior pituitary cells primed with estradiol. During the 48 h of estrogen priming (0.5 nM E_2), progesterone (50 nM) exposure began at time 0 (48 h of P_4), 12 (36 h of P_4), or 36 h (12 h of P_4) after estradiol was added to the cells. The cells were then washed twice with plain medium before 3 h of stimulation by GnRH (0.5 ng/ml). Cells exposed to vehicle (0.01% ETOH) or progesterone (100 nM) without estradiol priming for 48 h served as controls. a $P < 0.05$ as compared to estrogen-treated controls.

a prerequisite for progesterone-induced FSH release. Therefore, the effect of 5α-reductase inhibitor 4-MA (*N,N*-diethyl-4-methyl-3-oxo-4-aza-5α-androstane-17β-carboxamide, Merck, Sharp and Dohme, Rahway, NJ) [27, 28] on progesterone-induced FSH secretion was studied. Following 48 h of estrogen priming, cells were treated with 5α-reductase inhibitor 4-MA (100 nM) for 2 h. There was no effect on either basal or GnRH-stimulated FSH secretion. When cells were exposed to 4-MA along with progesterone for 2 h, the stimulatory effect of progesterone on GnRH-stimulated FSH secretion was antagonized ($P < 0.05$). There was no significant effect on basal FSH levels (Fig. 6). 4-MA itself, following 48 h of estrogen priming, stimulated both basal and GnRH-stimulated LH secretion ($P < 0.05$), (Table 1). When co-incubated with progesterone, 4-MA partially antagonized the elevation of both basal and GnRH-stimulated LH that was caused by progesterone.

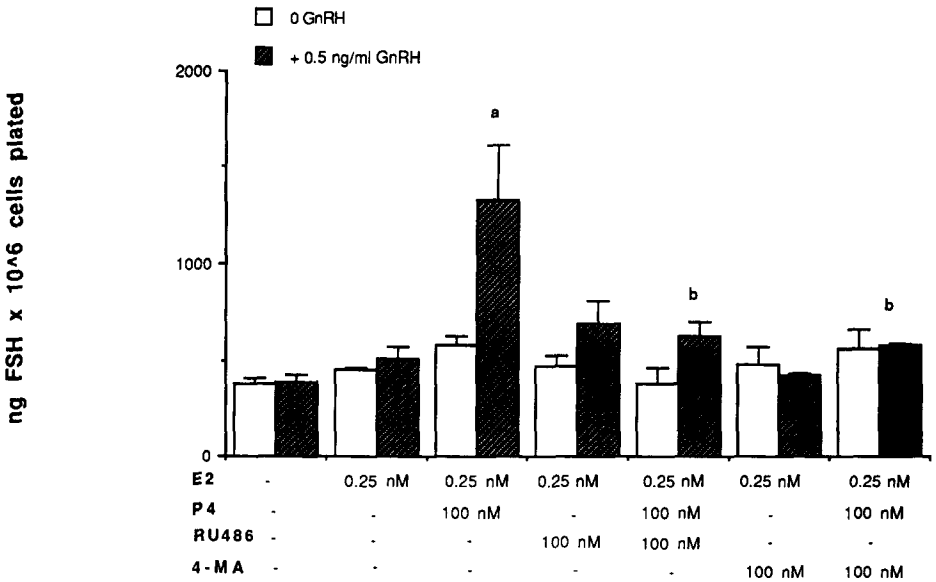

Fig. 6. Effect of the progesterone antagonist, RU486, and a 5α-reductase inhibitor, 4-MA, on progesterone-stimulated FSH secretion. Following 48 h of preincubation, cells were exposed to 48 h of estradiol (0.25 nM) where medium was changed and replenished every 24 h. Cells were then exposed to 2 h of vehicle (0.01% acetone), progesterone (100 nM), RU486 (100 nM), 4-MA (100 nM), progesterone + RU486 (100 nM each) or progesterone + 4-MA (100 nM each) followed by stimulation with GnRH (0.25 ng/ml) for 3 h. FSH in medium was quantified by RIA. **a** $P < 0.05$ as compared to estrogen-treated controls, **b** $P < 0.05$ as compared to estrogen–progesterone combined treatment.

DISCUSSION

The regulation of gonadotropin secretion by steroids is complex. Classically, estradiol has been considered to be solely responsible for the preovulatory surge of the gonadotropins. Early studies revealed that ovariectomy caused an elevation in gonadotropin secretion which could be suppressed by administration of estradiol. When ovariectomy was performed on the day before the preovulatory surge of gonadotropins, the surge was abolished. The surge could then be reinstated by administration of estradiol [29, 30]. The administration of estrogen antagonists [31, 32] and antibodies to estradiol [33] have also been effective in abolishing the gonadotropin surge. Recent work, however, has provided increasing evidence for a critical role for progesterone in the regulation of the gonadotropin surge [5, 6, 34–36]. The first elevation in serum progesterone occurs before the onset of the gonadotropin surge [37, 38] and has been shown to play a regulatory role on the estradiol-induced gonadotropin surge. The integrative effects of estradiol and progesterone on facilitation and suppression of gonadotropin secretion have been extensively reviewed [34–36]. However, the precise site(s) of action of progesterone is poorly understood. Since progesterone receptors have been localized in both hypothalamic tissue as well as in the anterior pituitary gland [12–16], the actions of progesterone could be at the central nervous system or at the level of the anterior pituitary gland, or may involve action at both sites. At the hypothalamus, progesterone stimulates the rapid release of GnRH [17, 18] and diminishes the degradation of GnRH [39, 40]. Progesterone effects on gonadotropin secretion can be either stimulatory or inhibitory depending on the time of administration. Elegant studies by Everett [41] demonstrated that progesterone would enhance the surge if administered just before the expected gonadotropin surge in normally cycling rats. If, on the other hand,

Table 1. Effect of RU486 and 4-MA on progesterone (P_4)-stimulated LH secretion[a]

Experimental conditions	ng LH/1 × 10^6 cells plated	
	Basal	GnRH-stimulated
Vehicle	306.8 ± 48.5*	627.5 ± 137.3*
E2	693.7 ± 53.1	1158.8 ± 190.1
E2 + P_4	1835.2 ± 35.1*	2018.6 ± 24.3*
E2 + RU486	1147.0 ± 217.4*	1095.5 ± 77.5
E2 + P_4 + RU486	475.4 ± 29.4**	1779.9 ± 316.4
E2 + 4-MA	1412.0 ± 124.7*	1995.8 ± 111.2*
E2 + P_4 + 4-MA	1206.8 ± 18.7*,**	1595.7 ± 31.8*,**

[a] Following 48 h of preincubation, anterior pituitary cells were exposed to 48 h of estradiol (0.25 nM) where medium was changed and replenished every 24 h. Cells were then exposed to 2 h of vehicle (0.01% acetone), progesterone (100 nM), RU486 (100 nM), 4-MA (100 nM), or progesterone + test compound (100 nM each) followed by stimulation with GnRH (0.25 ng/ml for 3 h). LH in medium was quantified by RIA.

*$P < 0.05$ as compared to estrogen-treated controls; **$P < 0.05$ as compared to estrogen–progesterone combined treatment.

progesterone was administered early in the morning of the day of the expected gonadotropin surge, the effect was to delay the onset of the surge. A model explaining this action of progesterone which involved both hypothalamic and pituitary sites of action was proposed by Mahesh and Muldoon in 1987 [35] and subsequently validated by Brann *et al.* in 1991 [42].

The major questions raised in this study were to determine if progesterone had a direct effect on the anterior pituitary in the regulation of gonadotropin secretion. If so, were progesterone receptors involved in such action. The role of 5α-reductase in the action of progesterone in the anterior pituitary was also investigated. The role of progesterone in the secretion of FSH was of particular interest in this study because very little information is available regarding FSH secretion as compared to LH secretion. Furthermore, progesterone has been reported to bring about FSH secretion in a number of species [5–9].

The results of this study confirmed previous work that estrogen priming was necessary for progesterone action because estrogens are required for progesterone receptor synthesis [43]. Furthermore, they also support previously reported results that estradiol treatment sensitized anterior pituitary cells to GnRH in the release of LH and FSH [19–23]. In addition to the above, progesterone treatment for 2–6 h in estrogen-primed anterior cells resulted in an increase in basal LH secretion and at 1–6 h in GnRH-stimulated LH release. Furthermore, progesterone treatment for 48 or 36 h resulted in a significant inhibition of GnRH-induced LH release. Those results agree with previously published results [19–23]. The progesterone-induced LH release or sensitization to GnRH in the release of LH appeared to be mediated through the progesterone receptor system because of the necessity of estrogen priming for progesterone action [43]. The progesterone antagonist RU486 can also act as a progesterone agonist in adult rat pituitary cell cultures in the absence of progesterone [44]. In this study the stimulatory effect of RU486 was observed in the presence of estrogen priming whereas in the absence of estrogen priming, it did not have any effect on basal or GnRH-stimulated LH release. The use of RU486 in the presence of progesterone attenuated the basal LH release induced by progesterone. Such an effect of RU486 has also been observed in the PMSG-primed immature female rat and the cycling adult rat [36, 45–47]. Finally, the 5α-reductase inhibitor had only minimal effects on progesterone-induced basal and GnRH-stimulated LH release. Even in the presence of the 5α-reductase inhibitor progesterone-induced basal and GnRH-stimulated LH release was significantly greater than that found in estrogen-primed dispersed anterior pituitary cells. The absence of any effects of the 5α-reductase inhibitor on progesterone-stimulated LH release in dispersed pituitary cells *in vitro* using a different dose [47] and in estrogen-primed ovariectomized rats *in vivo* has been demonstrated earlier [48]. The absence of the effects of 5α-reduction of testosterone on testosterone-induced LH release from dispersed pituitary cells has also been reported [49].

In this study the dose of estradiol used for priming the dispersed pituitary cells was 0.25 and 0.5 nM. In previous studies the circulating estradiol levels in the rat estrus cycle have been reported to be 0.02 to 0.07 nM during diestrus-I and 0.17 to 0.51 nM during proestrus [2, 50–52]. Thus the level of estradiol used was comparable to levels found at proestrus. Circulating levels of progesterone reported in the rat estrus cycle vary from 15–30 nM on diestrus-I and 127–287 nM on proestrus [2, 50–52]. The doses used in this study varying from 50–200 nM are well within this range with 50 nM levels near the early rise levels of proestrus. Immunoreactive GnRH levels have been reported to be 14 ± 1 pg/ml on estrus and 79 ± 11 pg/ml on proestrus in hypophysial–portal plasma [53]. In our study, the 0.5 ng/ml dose of GnRH was used because it brought about both an increase in LH and FSH release. The ED_{50} for LH and FSH release in estrogen-primed dispersed pituitary cells was 0.19 and 0.36 ng/ml, respectively. Considering the fact that GnRH is delivered continuously over a period of time via hypophysial–portal blood *in vivo* and is very rapidly degraded in the pituitary [39, 40], a one time addition of 0.5 ng/ml was considered reasonable.

Under the conditions of cell culture employed and the dose of GnRH used, the stimulation of FSH secretion by progesterone was less consistent than LH. Basal levels of FSH were stimulated by 50, 100 and 200 nM progesterone at the 3-h time point (Figs 2 and 3) and GnRH-stimulated FSH release in the presence of 50 nM progesterone in several experiments. This is not surprising because the relationship between FSH secretion after GnRH stimulation is not as consistent as with LH secretion. Furthermore,

in these experiments we attempted to use a dose of GnRH which could be used to study both LH and FSH release. Using a perifusion system, Kellom and O'Conner [54] have demonstrated that much larger quantities of GnRH are required for FSH release as compared to LH release.

A consistent inhibitory effect of progesterone on GnRH-stimulated FSH release was observed when 50, 100 and 200 nM progesterone were employed for 48 h (Fig. 4). Such an effect was also observed when the period of progesterone treatment was reduced to 36 or 12 h using the 50 nM dose of progesterone (Fig. 5). Thus the stimulatory effect of progesterone on FSH release was observed after a 3-h exposure whereas 12-h or longer periods led to inhibition of FSH secretion. These observations are consistent with the short and long-term effects of progesterone observed by Everett [41] and confirmed by several subsequent studies (reviewed in [32–34]).

In an experimental model proposed by Mahesh and Muldoon in 1987 [35] it was suggested that the stimulatory or inhibitory effect of progesterone or gonadotropin secretion depended upon the adequacy of estrogen priming. This hypothesis was validated by subsequent experiments of Brann *et al.* [42]. In the anterior pituitary, the administration of progesterone is able to bring about a reduction of occupied nuclear estrogen receptors [43, 55]. Whereas this may facilitate acute gonadotropin release by decreasing the acute inhibitory effect of estrogens on LH and FSH release on the pituitary [34, 42], the long-term effect would be a decrease in progesterone receptor synthesis [43] and interference with the enhancement of pituitary sensitivity to GnRH in the release of LH and FSH in response to estrogen [35]. This may be one of the complex mechanisms that may explain the differences observed in short-term and long-term effects of progesterone on gonadotropin secretion *in vivo* and in this study. The short-term stimulatory effect of progesterone on FSH secretion provides an explanation for progesterone-induced FSH release *in vivo* in the rat [5, 6] and other species [7–9].

The FSH-releasing action of progesterone appears to be mediated through the progesterone receptor because the progesterone antagonist RU486 prevented the GnRH-stimulated FSH release observed after progesterone treatment. Unlike the observations with LH release, RU486 by itself did not stimulate basal or GnRH-stimulated FSH release (Fig. 6). RU486 has also been found to inhibit FSH release in the PMSG-primed intact rat and the cycling adult rat [36, 45]. Of considerable interest was the finding that the suppression of pituitary 5α-reductase by the use of the 5α-reductase inhibitor 4-MA, blocked the effect of progesterone on FSH release (Fig. 6). The 5α-reductase inhibitor by itself did not have any effects on FSH release in estrogen-primed anterior pituitary cells. In the rat anterior pituitary the 5α-reductase activity apepars to be concentrated in the gonadotropes [56, 57]. It does not appear to play a significant role in the secretion of LH because 4-MA was unable to alter the suppressive effect of testosterone on LHRH-induced LH release in dispersed pituitary cell cultures [49, 58] and progesterone-facilitated LH release in response to GnRH [47]. These results suggest that 5α-reduction of progesterone is essential for its action of FSH release but not LH release from the anterior pituitary. These observations confirm the findings of Putnam-Roberts *et al.* [48] that 5α-reduction of progesterone appears to be essential for progesterone-induced FSH release *in vivo* in estrogen-primed ovariectomized immature rats.

In summary, this study demonstrates important modulatory effects of progesterone in the regulation of LH and FSH release in the anterior pituitary using dispersed pituitary cell monolayer cultures. Furthermore, it demonstrates that 5α-reduction of progesterone is important for progesterone-induced FSH release but not LH release by the anterior pituitary gland.

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