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ESTRADIOL AND PROGESTERONE EFFECTS ON RELATIVE LUTEINIZING HORMONE AND FOLLICLE STIMULATING HORMONE RELEASE INDUCED FROM SUPERFUSED ANTERIOR PITUITARY CELL CULTURES BY DEFINED LHRH PULSE REGIMENS

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Summary—These studies examined the capacity of estradiol and progesterone to modulate relative luteinizing hormone (LH) and follicle stimulating hormone (FSH) secretion from superfused anterior pituitary cells when stimulated with luteinizing hormone releasing hormone (LHRH) pulse regimens of specific amplitude, duration and frequency. There was particular interest in whether such steroid and LHRH treatments induced evidence of divergent LH or FSH secretion. Pituitaries were recovered from adult, 2 week ovariectomized rats and cultured for 48 h with collagen coated Cytodex microcarrier beads. Cultures were preincubated either with or without estradiol (1 or 10 nM) for 48 h and were subsequently incubated for 3, 6 or 12 h with 100 nM progesterone. All groups were then pulsed with 1 of 3 LHRH regimens; regimen 1 delivered 8 ng in a single 100 μl bolus once/h; regimen 2 divided the 8 ng dose of regimen 1 into 3 equal doses administered at 4 min intervals thereby maintaining the 8 ng mass of regimen 1 while extending the duration of exposure; regimen 3 was the same as regimen 2 except that the 3 equal doses were administered at a pulse frequency of 1 per 2 h rather than 1 per h thereby not only maintaining the duration of exposure as in regimen 2 but also reducing the pulse frequency.

1 nM estrogen alone for 48 h had no effect on LHRH stimulated LH release regardless of regimen; however, FSH was increased when hourly pulses of increased duration were applied (regimen 2). When estrogen was increased to 10 nM, regardless of regimen, LH was predominantly inhibited while FSH was unaffected. When 1 nM estrogen was followed by progesterone, both LH and FSH were elevated at 6 h progesterone in response to regimen 2; with 10 nM estrogen however, a divergent response was observed in that LH release was elevated at 6 h while FSH was elevated at 3 h in response to regimens 2 and 3. These results first of all confirm that progesterone in combination with estrogen is capable of exerting both inhibitory and stimulatory effects on gonadotropin secretion; secondly, these studies show that, as a direct pituitary effect, the LHRH regimen and the gonadal steroid milieu are capable of interacting to significantly influence the relative secretion of LH and FSH. The data therefore suggest that the divergent gonadotropin secretion seen in various physiological states *in vivo* is due likely in part to a combination of estrogen and progesterone priming in combination with the hypothalamic LHRH secretory pattern.

INTRODUCTION

Those factors controlling the relative release of luteinizing hormone (LH) and follicle stimulating hormone (FSH) are poorly understood. It is well documented that gonadotropin secretion is a consequence of pulsatile luteinizing hormone releasing hormone (LHRH) acting on gonadotrophs whose sensitivity to the releasing factor is heavily influenced by the *in vivo* gonadal steroid milieu existing at the time of stimulation [1–3]. Estradiol (E) and progesterone (P) have been demonstrated to feedback at the level of the pituitary in a positive [4–8] and negative [9–12] manner in modulating LHRH induced LH and FSH release [1, 13, 14]. In addition, the influence of P on LH secretion is dependent on E pretreatment both *in vivo* [15, 16] and *in vitro* [8, 11, 17]. P metabolites have been shown to divergently augment FSH secretion in the E primed rat and E is more effective than P in suppressing post ovariectomy serum LH [18]. Gonadal steroids are not alone in their capacity to differentially modulate LH

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and FSH secretion. *In vivo* studies with ovariectomized ewes [19] and Rhesus monkeys [20] have shown that the pattern of the LHRH stimulus also contributes to the divergence of gonadotropin secretion. Concerning pulsatile LHRH, in previous work we have shown that even in the absence of gonadal steroid supplementation, superfused anterior pituitary cells retain heightened proestrous responsiveness to pulsatile LHRH [14] and that the amplitude, duration and frequency of pulsatile LHRH play a significant role in modulating the relative proportions of LH and FSH which are secreted [21, 22]. While several studies examining the role of LHRH pulse patterns *in vitro* have been conducted, most report LH data only [16, 23, 24]. In addition, no one has reported steroid effects on both LH and FSH secretion under stimulation by different LHRH pulse regimens. Considering that fluctuations in gonadal steroids [25, 26] as well as LHRH [27] occur concurrently *in vivo*, we have undertaken the following studies with the objective of examining the combined effects of E, P and defined LHRH pulse patterns on relative LH and FSH secretion utilizing a superfusion system and microcarrier attached anterior pituitary cells.

EXPERIMENTAL

Animals

Adult female Sprague–Dawley rats (Harlan Inc., Indianapolis, IN) were housed with a lighting schedule of 14:10 (light–dark) with food and tap water available *ad libitum*. Ovariectomy was carried out under deep ether anesthesia; two weeks later anterior pituitaries were collected. Ovariectomized animals were employed so as to rule out endogenous steroid effects. The animal protocol was approved by the Institutional Committee on Animal Use before beginning the studies.

Cell dispersal

The cell dispersal procedure has been described previously [2]. Briefly, pituitaries were dispersed with 0.25% trypsin (Gibco 610-5090; Grand Island, NY) which was terminated with 0.1% lima bean trypsin inhibitor (Gibco T-9767). Cells were mixed with Cytodex III microcarrier beads (Sigma 3275; St Louis, MO) and cultured for 48 h prior to superfusion [1]. Cells were incubated in complete growth medium (CGM) which consisted of Dulbecco's Modified Eagle medium without phenol red (DMEM; Gibco 3000EB). CGM was supplemented with 5% horse serum (Sigma H7889) and 5% fetal bovine serum (Sigma F3010) which had been dextran charcoal treated to remove endogenous steroids prior to inclusion in culture media [28].

Experimental design

Steroids were added to cultures in 50 μl volumes of 0.001% ethanol (final ethanol concentration = 0.00005%). The concentrations and length of steroid treatments are outlined in Fig 1. The E and P levels employed were derived from the literature and have been utilized by

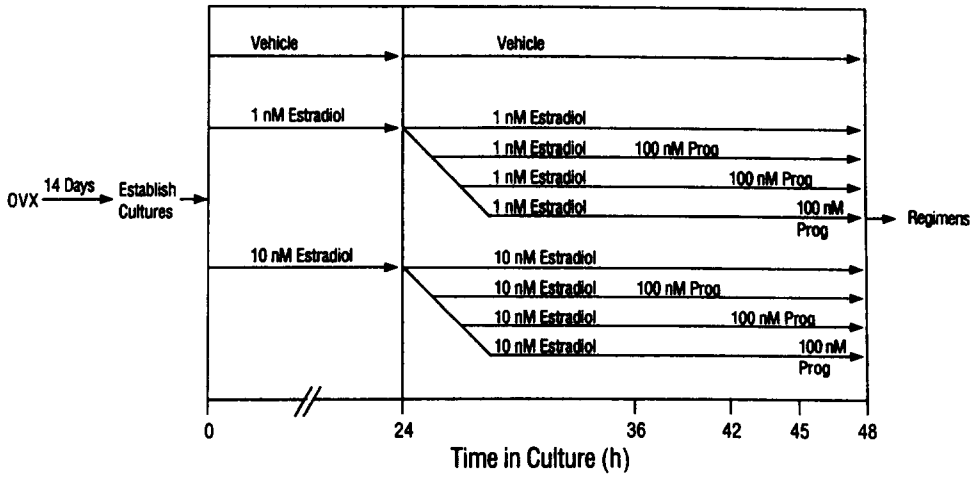

Fig. 1. Experimental design utilized to investigate the role of E and P on gonadotropin secretion. Dispersed anterior pituitary cells were incubated with either 1 or 10 nM E or vehicle for a total of 48 h. At 24 h, media were changed and again supplemented with E or vehicle. During the last 12 h of culture, groups ($n = 6$) were incubated with 100 nM P for 3, 6 or 12 h. At 48 h, cultures were stimulated with one of the three LHRH regimens described in Table 1.

other investigators in similar experimental conditions [6, 7, 29, 30]. Control cultures were incubated with CGM plus vehicle while test cultures were incubated with CGM plus vehicle containing either 1 or 10 nM E (Sigma E8875) for 48 h. Prior to superfusion, P (Sigma P0130) treated cultures were incubated with 100 nM P for either 3, 6 or 12 h; these time points were chosen in order to mimic the rising P phase associated with the afternoon and evening of proestrus in the rat [31, 32].

Superfusion system and LHRH regimens

The physical configuration of the superfusion system has been described previously [1, 3, 33]. Three pituitaries dispersed as a pool were used in each column and six columns were run per experimental group. After 48 h of culture, bead attached cells were loaded into columns and superfused with DMEM; serum was eliminated from this medium due to its potential for degradation of LHRH [34]. Columns were superfused at a flow rate of 0.125 ml/min and 1 ml fractions were collected. LHRH stimulation was not applied for the first hour to allow cells to reach stable basal release of gonadotropins. At the beginning of the second hour, columns were pulsed with one of three LHRH regimens (Table 1). Regimen 1 consisted of 8 ng LHRH (Sigma L-0507) in 100 μl phosphate buffered saline plus 0.1% gel (PBS + gel) injected once hourly at the beginning of hours two and three. At the beginning of the fourth hour, a 100 ng pharmacological bolus of LHRH was administered to test responsiveness at the end of each experiment; because an entirely different mass of LHRH was involved, these 100 ng data have not been included in statistical evaluations. Regimen 2 was designed in order to extend the duration of exposure; a total of 8 ng LHRH was divided into three individual doses of 2.7 ng; these doses were separated by 4 min and were injected into the columns at the beginning of hours two and three. Assessment of LHRH elution profiles from the column verified that such application achieved the intended increase in duration of exposure and elution profiles with these regimens have been verified in previous publications [21]. Regimen 3 was the same as regimen 2 except that the interval between pulses was increased from 1 h to 2 h; this regimen therefore employed a pulse interval of 2 h rather than 1 h. In all regimens LHRH was applied in brief, well defined pulses in order to mimic the established pulsatile characteristics of *in vivo* LHRH secretion [27, 35]. Excepting the 100 ng termination bolus of the experiment, neither regimen applied LHRH beyond the second exposure phase in order to avoid potential LHRH priming effects. LHRH recovery from the superfusion system has been shown to be 91–95% [1, 21].

DNA assay and RIA

With the completion of each experiment, the cells were removed from the microcarrier beads with collagenase (Sigma 3130). DNA assays were conducted using a modification of the Hoescht 33258 assay [36] as described previously [37]. LH and FSH were estimated by RIA as previously described utilizing reagents from the National Pituitary Hormone Distribution Program; results were expressed in terms of RP-1 [2, 25, 33, 34].

Analysis of data

LH and FSH values obtained from column fractions were divided by DNA values obtained from the same columns. Secretory profiles expressed as ngLH or FSH/ngDNA were plotted for each column utilizing Lotus Symphony spread sheets and gonadotropin pulses were identified with the PC Pulsar computer program [38]; the program criteria were set such that, with a one point pulse, the hormone concentration was required to be 3.8 assay standard deviations greater than the baseline LH or FSH concentration $G(1) = 3.8$; the criteria for 2, 3, 4 and 5 point pulses were $G(2) = 2.6$, $G(3) = 1.9$, $G(4) = 1.5$ and $G(5) = 1.13$, respectively [21, 39]. These criteria identified pulses which were also recognized upon

Table 1. Regimens utilized to study the effects of LHRH pulse characteristics on the relative secretion of LH and FSH from estrogen primed and progesterone treated superfused anterior pituitary cell cultures

	GnRH (ng) injected into superfusion column in 100 μl					
Regimen No.	1h	2h	3h	4h	5h	6h
1	0	8	8	100	0	0
2	0	2.7–2.7–2.7 4 min apart	2.7–2.7–2.7 4 min apart	100	0	0
3	0	2.7–2.7–2.7 4 min apart	0	2.7–2.7–2.7 4 min apart	0	100

All masses of LHRH were added as single 100 μl volumes at the indicated time points in a vehicle of 0.01 M NaH_2PO_4 plus 0.1% gelatin.

visual inspection of plotted data. Areas beneath pulses were quantified as mm^2 by measurement with a Jandel digitizer and software (Corte Madera, CA). Significant differences in pulsatile responses were identified with Duncan's Multiple Range test subsequent to ANOVA.

RESULTS

Effects of E alone on LH and FSH secretion

Pretreatment with 1 nM E alone had no significant effect on LH secretion with any LHRH regimens tested when compared to controls [Figs 2, 3 and 4(A) and (B)]. FSH release was unaffected by 1 nM E pretreatment when pulsed with an 8 ng bolus [regimen 1; Fig. 2(C) and (D)] or when the pulse duration was extended and administered once per 2 h [regimen 3; Fig. 4(C) and (D)]; however, when stimulated hourly with an extended pulse duration (regimen 2), FSH was significantly elevated when compared to controls [Fig. 3(C) and (D), $P < 0.01$]. Pretreatment with 10 nM E significantly reduced LH when stimulated hourly with an 8 ng bolus [regimen 1; Fig. 2(E), $P < 0.05$; and (F), $P < 0.01$] as well as at pulse 1 of both regimen 2 [Fig. 3(E)] and regimen 3 [Fig. 4(E)]. 10 nM E alone had no significant effect on FSH secretion with any regimens tested.

Combined effects of 1 nM E and 100 nM P on LH and FSH

The responses to pulsatile LHRH in cells pretreated with 1 nM E and subsequently 100 nM P were compared to both controls (no steroid) and to cultures incubated with E alone. Significant effects were as follows. A 3 h incubation with P prior to stimulation with a single 8 ng pulse (regimen 1) resulted in significantly reduced LH compared to controls and E alone [Fig. 2(A) and (B), $P < 0.01$]. Increasing P to 6 h caused LH secretion to be higher than at 3 h but significantly reduced at the second LHRH pulse as compared to controls and E alone [Fig. 2(B), $P < 0.01$]. At 12 h, P again resulted in significantly less LH secretion compared to controls and 1 nM E alone [Fig. 2(A) and (B), $P < 0.01$]. Within the P treated groups, at the first LHRH pulse, 1 nM E followed by 6 h P released significantly greater LH than 3 or 12 h P [Fig. 2(A), $P < 0.01$]. At the second LHRH pulse, 6 and 12 h P released significantly greater LH than 3 h P [Fig. 2(B), $P < 0.01$ at 6 h; $P < 0.05$ at 12 h]. FSH was significantly reduced by 3 h P at the first pulse of regimen 1 compared to controls and at the second pulse compared to controls and 1 nM E alone [Fig. 2(C) and (D); $P < 0.05$]. With 6 h P, both the first and second LHRH pulses resulted in significantly less FSH than controls [Fig. 2(C); $P < 0.01$; and (D); $P < 0.05$]. Incubation with 12 h P released significantly less FSH than controls and 1 nM E alone at the second LHRH pulse [Fig. 2(D); $P < 0.01$]. Within the P treated groups, at the second LHRH pulse, both 3 and 6 h P resulted in significantly greater FSH than 12 h P [Fig. 2(D); $P < 0.01$].

The effect of 8 ng LHRH pulses of extended duration at hourly intervals (regimen 2) was next examined. At the first LHRH pulse, pretreatment with 1 nM E followed by 3 h P resulted in significant reduction of LH compared to controls and 1 nM E alone [Fig. 3(A); $P < 0.01$]. At the second pulse, LH release was not significantly changed compared to controls and 1 nM E alone [Fig. 3(B); $P < 0.01$]. With 12 h P, LH was significantly reduced at the first LHRH pulse compared to controls and 1 nM E alone [Fig. 3(A); $P < 0.01$]. Within the P treated groups, at both LHRH pulses, 6 h P released significantly greater LH [Fig. 3(A) and (B); $P < 0.01$]. At 3 h P, FSH release was significantly reduced compared to 1 nM E alone [Fig. 3(C) and (D); $P < 0.01$]. P for 6 and 12 h resulted in significantly greater FSH compared to controls [Fig. 3(C) and (D); $P < 0.01$]. Within the P treated groups, 6 or 12 h P released significantly greater FSH compared to 3 h [Fig. 3(C) and (D); $P < 0.01$].

The effect of 8 ng LHRH pulses of extended duration as well as reduced frequency (regimen 3) subsequent to incubation with P was next examined. At 3 h P, LH release was significantly reduced compared to controls and 1 nM E alone [Fig. 4(A) and (B); $P < 0.01$]. At the second LHRH pulse, 6 h P significantly elevated LH compared to 1 nM E alone [Fig. 4(B); $P < 0.05$]. Incubation of cells with P for 12 h resulted in significantly less LH at the first LHRH pulse compared to controls and 1 nM E alone [Fig. 4(A); $P < 0.01$], as well as significantly less than 1 nM E alone at the second LHRH pulse [Fig. 4(B); $P < 0.05$]. Within the P treated groups, 6 h P resulted in significantly greater LH than 3 or 12 h P [Fig. 4(A) and (B); $P < 0.01$]. P for 3 h had no effect on FSH; however, at pulse 1, 6 h P released significantly greater FSH than controls and 1 nM E alone [Fig. 4(C) and (D); $P < 0.01$]. P for 12 h resulted in significantly less FSH secretion than controls at the

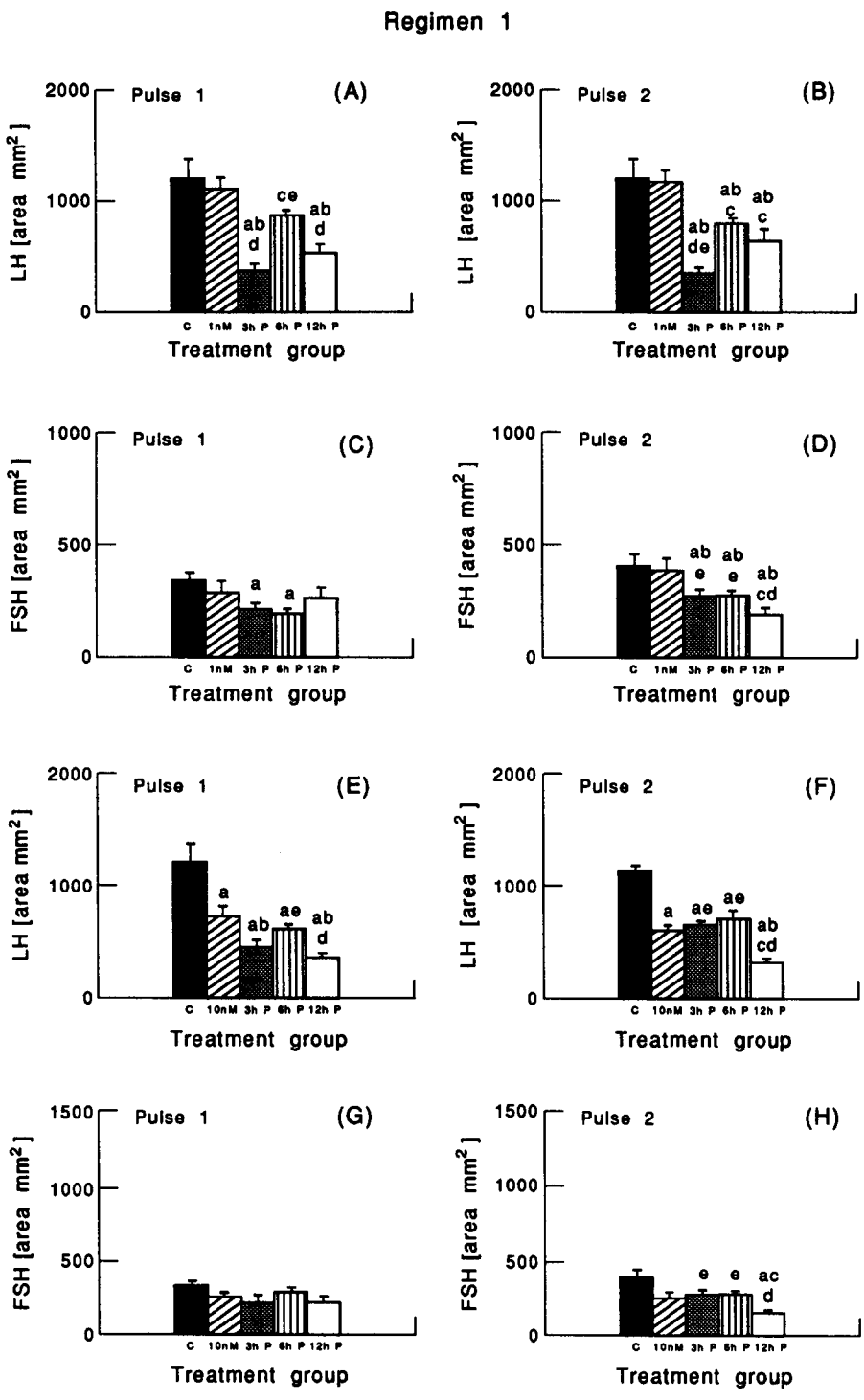

Fig. 2. Effects of 1 nM (panels A–D) and 10 nM (panels E–H) E and 100 nM P on LH and FSH secretion in response to 8 ng LHRH pulses of brief duration (regimen 1). Anterior pituitaries were recovered from two week ovariectomized rats and cultured for 48 h with indicated E levels. During the last 12 h of the culture period, groups of cultures ($n = 6$) were incubated with 100 nM P for 3, 6 or 12 h. Cultures were then exposed to indicated LHRH regimen. The graphed data represent the mean ± SE of 6 individual columns; three anterior pituitaries dispersed as a pool were used in each column. The letters a, b, c, d, e = significantly different from control, E alone, E + 3 h P, E + 6 h P and E + 12 h P, respectively.

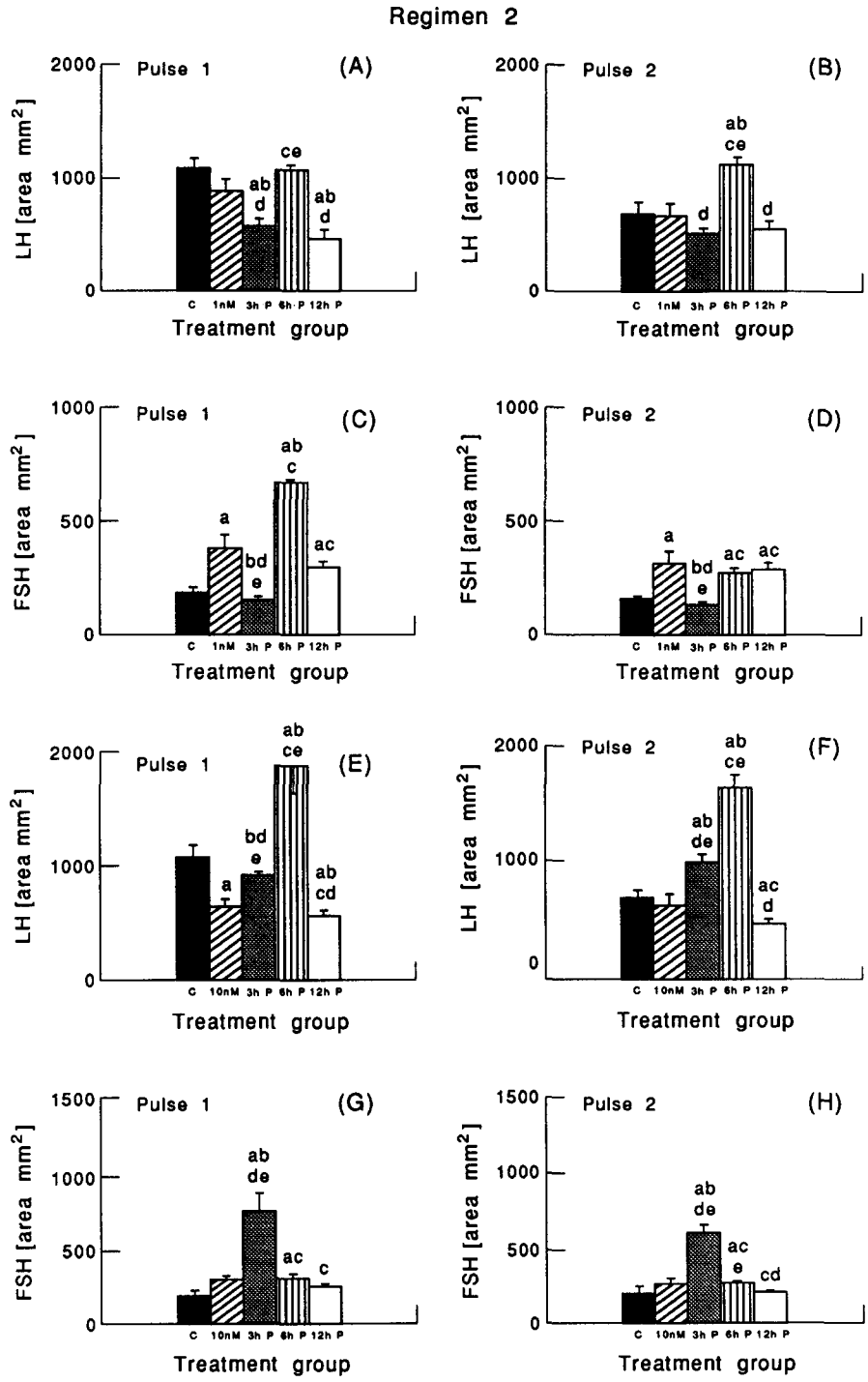

Fig. 3. Effects of 1 nM (panels A–D) and 10 nM (panels E–H) E and 100 nM P on LH and FSH secretion in response to 8 ng LHRH pulses of extended duration (regimen 2). Remainder of legend reads as in Fig. 2.

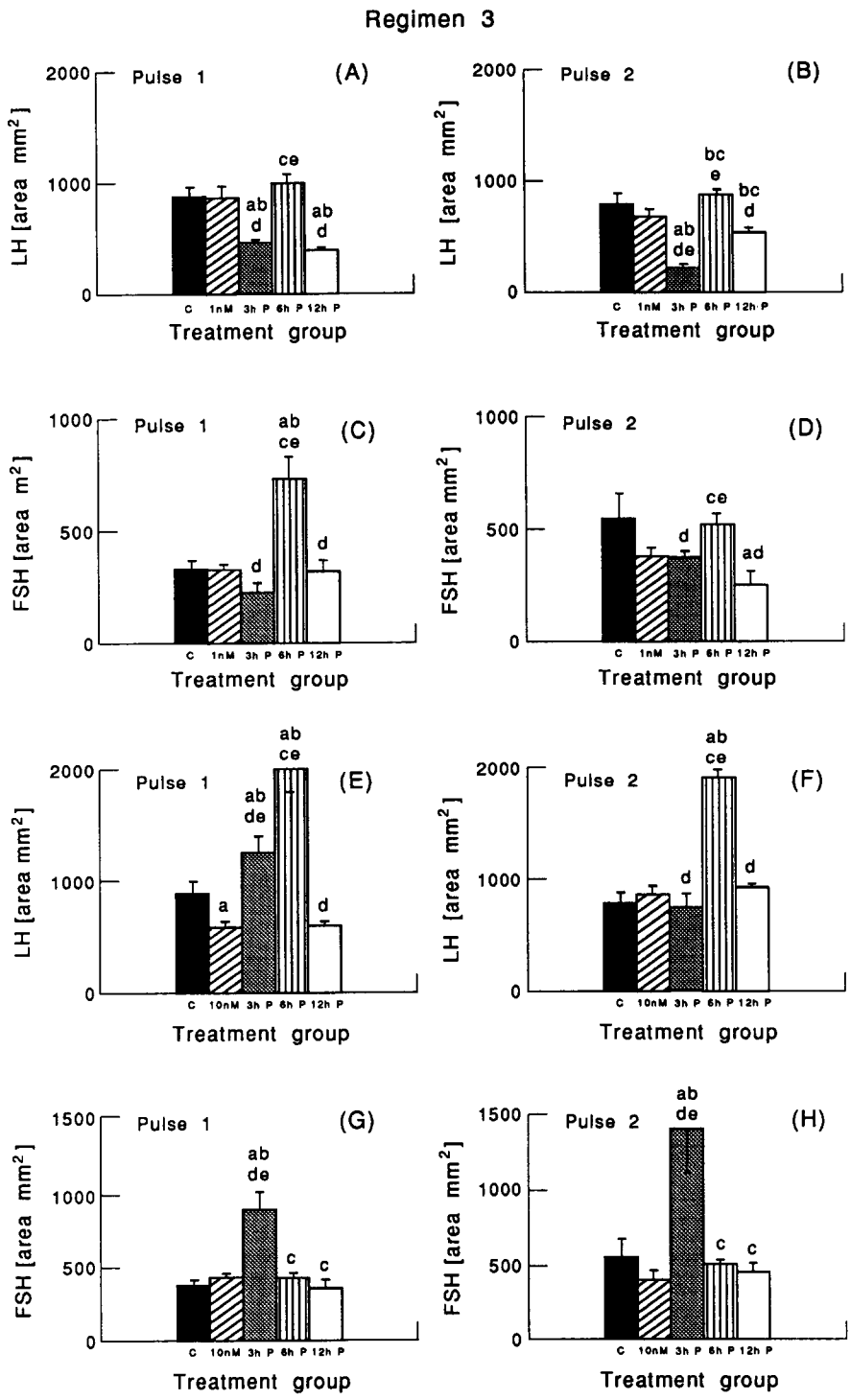

Fig. 4. Effects of 1 nM (panels A–D) and 10 nM (panels E–H) E and 100 nM P on LH and FSH secretion in response to 8 ng LHRH pulses of extended duration and reduced frequency (regimen 3). Remainder of legend reads as in Fig. 2.

second LHRH pulse [Fig. 4(D); $P < 0.05$]. Within the P treated groups, 6 h P resulted in significantly greater FSH than 3 or 12 h P [Fig. 4(C); $P < 0.01$; and (D); $P < 0.05$].

Combined effects of 10 nM E and 100 nM P on LH and FSH

The responses to pulsatile LHRH in cells pretreated with 10 nM E and subsequently 100 nM P were compared to both controls (no steroid) and to cultures incubated with E alone. Significant differences were as follows. Stimulating cells with a single 8 ng LHRH pulse (regimen 1) after 10 nM E significantly reduced LH at 3, 6 and 12 h P compared to controls [Fig. 2(E) and (F); $P < 0.01$]. Within the P treated groups, 6 h P resulted in significantly greater LH release than 12 h at the first LHRH pulse [Fig. 2(E); $P < 0.01$]; at the second LHRH pulse, both 3 and 6 h P resulted in significantly greater LH release than 12 h [Fig. 2(F); $P < 0.01$]. Neither 3, 6 nor 12 h P had any effect on FSH release at the first 8 ng pulse [Fig. 2(G)]; at the second LHRH pulse, only 12 h P caused a significant reduction in FSH compared to controls [Fig. 2(H); $P < 0.01$]. Within the P treated groups, 3 or 6 h P released significantly more FSH as compared to 12 h P at the second LHRH pulse [Fig. 2(H); $P < 0.05$ at 3 h; $P < 0.01$ at 6 h].

Incubation of cells with 3 h P prior to stimulating with 8 ng LHRH pulses of extended duration (regimen 2) resulted in significantly greater LH release than with 10 nM E alone at the first LHRH pulse [Fig. 3(E); $P < 0.01$] and significantly greater than controls or E alone at the second LHRH pulse [Fig. 3(F); $P < 0.05$]. LH release was significantly greater at 6 h than controls and E alone at both LHRH pulses [Fig. 3(E) and (F); $P < 0.01$]. A 12 h P incubation resulted in significantly less LH compared to controls [Fig. 3(E) and (F); $P < 0.05$]. Within the P treated groups, 6 h P resulted in significantly greater LH release than 3 or 12 h P [Fig. 3(E) and (F); $P < 0.01$]. At 3 h P, FSH release was significantly increased compared to controls and 10 nM E alone [Fig. 3(G) and (H); $P < 0.01$]. The 6 h P incubation also significantly increased FSH release compared to controls [Fig. 3(G) and (H); $P < 0.01$]. Within the P treated groups, 3 h P released significantly greater FSH than 6 or 12 h P [Fig. 3(G) and (H); $P < 0.05$].

Stimulating cells with 8 ng LHRH pulses of extended duration at a frequency of 1 pulse per 2 h (regimen 3) after 3 h P significantly elevated LH release at the first LHRH pulse as compared to controls and 10 nM E alone [Fig. 4(E); $P < 0.01$]. With 6 h P, LH release was significantly greater at both the first and second LHRH pulses compared to controls and 10 nM E alone [Fig. 4(E) and (F); $P < 0.01$]. Within the P treated groups, 6 h P resulted in significantly greater LH release compared to the 3 or 12 h P incubation [Fig. 4(E) and (F); $P < 0.01$]. The combination of 10 nM E and 3 h P followed by stimulation with regimen 3 resulted in the highest FSH secretion seen in these studies. At both the first and second LHRH pulses, this particular combination resulted in significantly greater FSH release than controls or 10 nM E alone [Fig. 4(G) and (H); $P < 0.01$]. Within the P treated groups, 3 h P resulted in significantly greater FSH release than either 6 or 12 h P [Fig. 4(G) and (H); $P < 0.01$]. Therefore, under stimulation by regimen 3, LH and FSH exhibited divergent temporal responsiveness to P in that maximum stimulation was observed at 6 and 3 h, respectively.

DISCUSSION

Gonadal steroids play a decisive role in the modulation of gonadotropin secretion by exerting effects at both the hypothalamic and pituitary level. At the pituitary level, it has been shown that both E and P are capable of exerting positive [4–8] and negative [9–12] effects on gonadotropin secretion. It has also been documented that P effects are largely dependent upon E pretreatment both *in vivo* [15, 16] and *in vitro* [8, 11, 17]. Previous work in this laboratory [14, 21, 22] has shown that LHRH delivered in regimens of specific amplitude and duration is capable of significantly modulating the relative amounts of LH and FSH secreted by cultured gonadotrophs in the absence of steroid supplementation. Because no previous studies have examined gonadal steroid effects on both LH and FSH secretion under stimulation by different LHRH pulse regimens, the goal of this work was to utilize superfused anterior pituitary cells to study the role of E and P in modulating the relative secretion of LH and FSH under stimulation by pulsatile LHRH of defined characteristics.

Concerning the control cultures which received LHRH only with no steroids, there was no significant difference at pulse 1 or 2 in the LH [Fig. 2(A), (B), (E) and (F)] or FSH [Fig. 2(C),

(G), (D) and (H)] response to 8 ng LHRH delivered as a single pulse (regimen 1). In response to 8 ng LHRH delivered with extended duration at a pulse interval of 1 h (regimen 2), LH was significantly suppressed at pulse 2 in comparison to pulse 1 [Fig. 3(A), (B), (E) and (F)] while FSH was not significantly affected [Fig. 3(C), (D), (G) and (H)]. On the other hand, FSH in response to 8 ng LHRH delivered with extended duration at a pulse interval of 2 h (regimen 3) was significantly stimulated at pulse 2 [Fig. 4(C), (D), (G) and (H)] while LH was not significantly affected [Fig. 4(A), (B), (E) and (F)]. It would therefore appear that even in the absence of gonadal steroids, the LHRH pulse regimen alone can significantly influence the relative secretion of LH and FSH with FSH being sensitive to increased pulse duration and decreased frequency.

Incubation of cells with 1 nM E alone for 48 h prior to stimulating with LHRH had no effect on LH release while FSH release was elevated when hourly pulses of extended duration were applied. Increasing the E concentration to 10 nM caused an inhibition of LH release while having no effect on FSH. It appears then that E can differentially regulate LHRH stimulated gonadotropin secretion and its ability to do so is dependent on both the concentration of the steroid as well as the pattern of the LHRH stimulus. Both inhibitory [29, 40, 41] and stimulatory [42–44] E effects with *in vitro* systems of pituitary cells recovered from ovariectomized rats have been reported. A biphasic effect of E in pituitary cultures of rats and monkeys has been observed [7]. Differential effects of E, in that the LH response to LHRH is more susceptible to negative feedback effects as compared to FSH, have been shown [30]. Thus, the differential effects of E on LH and FSH secretion in these studies most likely result from the combined effects of the LHRH stimulus superimposed upon the direct modulation of pituitary gonadotropin secretory capacity by E.

At pulse 1, under the influence of 1 nM E priming, there was no significant stimulation of LH by P when compared to control response with regimens 1, 2 or 3 [Figs 2(A), 3(A) and 4(A)]; with FSH, P stimulated significant increase in FSH at 6 h in response to pulse 1 of regimens 2 and 3 [Figs 3(C) and 4(C)] but not regimen 1 [Fig. 2(C)]. Under the influence of 10 nM E priming, P had a tendency to suppress LH in response to regimen 1 [Fig. 2(E)] while in response to regimens 2 and 3, there was a tendency to stimulate LH at 6 h [Figs 3(E) and 4(E)]; with FSH, P had no significant effect on response to regimen 1 [Fig. 2(G)] while the response to regimens 2 and 3 [Figs 3(G) and 4(G)] was stimulated at 3 h as opposed to the previously observed 6 h with LH. At pulse 2, essentially the same trends emerged and it was noted that the 3 h P effect on the FSH response to regimen 3 was augmented even further [Fig. 4(H)]. It would appear then that the level of E pretreatment plays a strong role in modulating a divergent LH and FSH response to pulsatile LHRH of increased duration and reduced frequency (regimen 3); further, it appears that P effects are time dependent and may be either stimulatory or inhibitory depending on the length of exposure. A time dependent biphasic effect of P has also been reported *in vivo* [45] in which P first inhibited, then stimulated and with time again inhibited LH secretion.

The effects of gonadal steroids and LHRH regimens utilized in these studies on gonadotropin secretion could be partially modulated through mRNAs for the LH and FSH subunits. E has been shown to inhibit both LH [46, 47] and FSH [48, 49] beta subunit levels in rats. In addition, LH [50] and FSH [51] beta subunit concentrations have been shown to fluctuate in patterns similar to LH and FSH secretion during the estrous cycle of the rat. Variations in LHRH concentrations [52, 53] have been shown to differentially regulate LH-β subunit mRNA in static monolayer cultures, while changing LHRH frequency [54] as well as durations and amplitudes [55] differentially regulate LH-β mRNA *in vivo* in male rats.

In summary, we have shown in previous studies that LHRH pulse regimens applied to pituitary cells derived from cycled rats can exert a significant effect on divergent secretion of LH and FSH [14, 21, 22]. The current studies not only confirm that P, depending on dose and E milieu, may have stimulatory or inhibitory effects on gonadotropin secretion but extend these observations to include the fact that the LHRH milieu also is an integrative factor in modulating the relative levels of LH and FSH which are ultimately secreted. The changing gonadal steroid secretory pattern encountered during the estrous cycle may therefore not only influence gonadotropin secretion through altered pituitary LHRH responsiveness but also through altered LHRH pulse pattern. In these studies, maximum FSH secretion was seen in E

primed, progesterone treated cells when stimulated with an LHRH regimen of increased duration and reduced frequency. It may be then that the relative proportions of LH and FSH which are secreted by the pituitary are not only significantly influenced by the E and P exposure experienced by the pituitary but also by the hypothalamic LHRH secretory pattern.

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