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Estrogen and Progesterone Affect Electrically Induced Release of Luteinizing Hormone and Prolactin in Macaques[1] (41148)

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Abstract. We studied the effects of quasiphysiological regimens of estradiol-17β (E) and progesterone (P) on luteinizing hormone (LH) and prolactin release induced by electrical stimulation (ES) of the mediobasal hypothalamus (MBH) in ovariectomized monkeys. Monkeys with electrodes implanted in the MBH were treated with Silastic implants containing no steroid, E, or E plus P at 6, 24, or 30 hr prior to ES of the MBH. The following combinations were tested: 2E implants for 24 hr, 2E plus P for 24 hr, 1E for 30 hr, 1E plus P for 30 hr, 1E for 30 hr plus P for 6 hr, 2E for 30 hr, 2E for 30 hr plus P for 6 hr, empty implants. Blood was collected immediately before, during, and after stimulation. The serum was assayed for E, P, LH, and prolactin. With empty implants, the mean serum E concentration was 14 ± 2 pg/ml. This rose to 116 ± 14 for one E implant and to 224 ± 2.3 for two. One P implant raised the mean serum P concentrations from 0.30 ± 0.03 to 3.08 ± 0.21 ng/ml. The effect of E on serum LH varied among regimens—either no significant effect or reduction before, during, and after ES. Progesterone treatment significantly elevated LH concentrations before, during, and after ES. Prior to ES, serum prolactin concentrations of controls and E-treated animals were not significantly different. During ES the pattern of prolactin release was related to E dose. After ES, estrogen significantly affected both the mean concentration and rate of decline of serum prolactin. Progesterone had no significant effect on prolactin concentrations. These data show that, in the presence of E, P can facilitate LH release under both basal conditions and during ES.

The role of progesterone (P) in regulating luteinizing hormone (LH) and prolactin secretion during the menstrual cycle of the rhesus macaque is not clear. It either facilitates or inhibits LH secretion; the effect depends on the physiological state of the animal at the time of administration and the regimen of administration. Injection of P into intact female monkeys on Days 2–10, 8–16, or 8–22 of the cycle blocks the preovulatory LH surge (1); administration via silicone implants during Days 3–9 of the cycle also blocks estrogen-induced LH surges (2). In contrast, administration of P simultaneously with estradiol-17β (E) to ovariectomized females hastens the onset of LH surges compared with monkeys treated with E alone (2). Dierschke *et al.* (3) found that simultaneous injection of P with E blocked the ability of E to induce an LH surge in ovariectomized monkeys; however, when P was given 12 hr after E, the LH surges were advanced in some animals. Advancement of the LH surge by P injection also reportedly occurs in intact female monkeys (4). Helmond *et al.* (5) showed that P accelerates the LH surge induced by E and affects peak levels of LH released in respone to E.

The ability of P to advance the LH surge suggests that the small periovulatory rise of P may affect the character of the preovulatory LH surge in the primate. This suggestion is supported by the observation that small doses of P act within 4 hr *in vivo* to amplify the response of the estrogen-primed pituitary to exogenous gonadotropin-releasing hormone in the woman (6).

Gonadal hormones also may influence prolactin in rhesus macaques. Whereas changing levels of estrogen have no apparent effect on serum prolactin levels during the menstrual cycle (7) serum prolactin levels are lower in long-term ovariectomized monkeys than in intact females (8,

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9). Moreover, lesions in the mediobasal hypothalamus (MBH) elevate blood prolactin levels in intact but not ovariectomized monkeys (10), and release of prolactin induced by local electrical stimulation (ES) of the hypothalamus can be enhanced by treatment with E for 72 hr prior to stimulation (11). These observations suggest that ovarian steroids may modulate prolactin secretion in monkeys.

The main purpose of the study reported here was to determine if exposure of the hypothalamic–hypophyseal system to regimens of E and P, approximating those occurring during the periovulatory period, facilitates LH and prolactin release in response to ES of the MBH. Monkeys with electrodes implanted in the MBH were treated with either no steroid, E, or E plus P prior to ES. The response was quantified through measurement of blood levels of LH and prolactin prior to, during, and after stimulation.

Materials and Methods. The nine adult rhesus macaques (*Macaca mulatta*) had been ovariectomized at least 1 month earlier. Bilateral monopolar electrodes were placed into the brain of each female with procedures described elsewhere (12). Two pairs of electrodes were placed in the arcuate-ventromedial nuclear (MBH) region and either one or two pairs were placed in the rostral hypothalamus. The rostrally implanted electrodes were not used in this study. The assembly base was then permanently fixed to the calvarium with dental acrylic and anchor screws. Each monkey was returned to its cage for at least 2 weeks of recovery.

The animals were then assigned to one of three experiments; each compared the effects of no steroid, E alone, and E plus progesterone on basal LH and prolactin levels, and on the changes in circulating levels of these hormones in response to ES of the MBH. The experiments differed in levels of steroids and in length of treatment prior to ES. Within an experiment (A–C; Table I) each animal was subjected to all three or four regimens. The number of animals and trials varied among experiments. A trial was defined as a single treatment of an animal with a stated regimen of steroid and ES. Experiment A used three

TABLE I. REGIMENS OF TREATMENT OF OVARIECTOMIZED MONKEYS WITH EITHER ESTRADIOL OR ESTRADIOL PLUS PROGESTERONE PRIOR TO LOCALIZED ELECTRICAL STIMULATION OF THE MEDIOBASAL HYPOTHALAMUS

Experiment	Regimen[a]	Mean serum concentrations	
		E (pg/ml)	P (ng/ml)
A	1. Empty implants (3)	15 ± 2	0.31 ± 0.04
	2. 2E implants for 24 hr (5)	220 ± 15	—
	3. 2E implants plus 1P implant for 24 hr (6)	228 ± 25	3.47 ± 0.38
B	1. Empty implants (5)	13 ± 3	0.28 ± 0.05
	2. 1E implant for 30 hr (8)	115 ± 11	—
	3. 1E implant plus 1P for 30 hr (8)	123 ± 19	3.02 ± 0.21
	4. 1E implant for 30 hr plus 1P implant for last 6 hr (8)	110 ± 13	3.80 ± 0.32
C	1. Empty implants (5)	15 ± 4	0.32 ± 0.03
	2. 2E implants for 30 hr (8)	215 ± 16	—
	3. 2E implants for 30 hr plus 1 P implant for last 6 hr (8)	233 ± 38	3.70 ± 0.40

[a] Steroids were administered via Silastic capsules placed subcutaneously in the subscapular region prior to the onset of electrical stimulation (ES). The hours indicated refer to the number of hours the implants were in place prior to the onset of stimulation. Serum concentrations of estradiol-17β (E) and progesterone (P) were measured in samples taken immediately before stimulation. The numbers in parentheses indicate the number of trials in which that regimen was tested. Some trials with empty implants served as controls for more than one experiment. Thus, the total separate number of trials with empty implants was 11. The intervals between insertion of empty implants and ES were the same as between insertion of steroid filled implants and ES.

animals for a total of 14 trials. Experiment B used five animals for a total of 26 trials. Experiment C used five animals for a total of 21 trials.

The steroids were administered with 3- or 4-cm long Silastic capsules as described elsewhere (2). Before implantation, the capsules were incubated overnight in 1% bovine serum albumin. Immediately before implantation, they were rinsed in antiseptic and then in sterile saline. The capsules were inserted beneath the skin of the subscapular region. During the treatment regimen, the capsules were inserted 30, 24, or 6 hr before ES. The treatment regimens were designed to test the effect of P either under conditions where there was insufficient E to induce an LH surge (one E implant) or under conditions where treatment with P was expected to hasten the onset of an LH surge in response to E (two E implants). In the second case ES was conducted 3 to 6 hr prior to the expected time of onset of the LH surge in order to improve the chances of detecting an effect of P on the LH release induced by ES. The performance of each implant was evaluated through assays of serum samples for E and P concentrations. With empty implants, the mean serum E concentration was 14 ± 2 pg/ml. This rose to 116 ± 14 pg/ml with one E implant and to 224 ± 23 pg/ml with two, ignoring the possible effects of P implants. One P implant raised the mean serum P concentration from 0.30 ± 0.03 to 3.08 ± 0.21 ng/ml, ignoring possible effects of E implants.

One day before each stimulation trial, the monkey was placed in a primate chair. The Silastic capsules were then inserted, and the animal was placed inside an electrostatically shielded, sound-muffled chamber. Approximately 24 hr (at 1000 hr) or 30 hr (at 1400 hr) after insertion of the first capsule, biphasic current (0.25 mA, 5 mV, 1-msec pulse width; 100 Hz) was delivered bilaterally to the MBH region at 30-sec on–off intervals for 30 min (12). Current was passed simultaneously across both sets of electrodes in this region.

A catheter was inserted into the saphenous vein approximately 1.5 hr prior to the onset of ES. Blood samples (2 ml) were collected 60, 30, and 1 min before ES; 5, 15, and 30 min into ES; and 15, 30, and 60 min after the 30-min stimulation period.

Immediately after each ES regimen, the steroid implants were removed and the monkeys were returned to their cages for a rest of at least 2 weeks before the next trial.

Blood samples were left to clot overnight at 4° and were centrifuged at 1000*g* for 30 min. Serum was then withdrawn and stored at −20° until LH, prolactin, E, and P were measured by radioimmunoassay. A partially purified preparation of rhesus adenohypophysis (LER-M-907D) with a biological activity of 0.025 NIH-LH-S1 units/mg and a follicle-stimulating hormone biological activity of 0.26 NIH-FSH-S1 units/mg was used as a standard for the LH assays. Assay procedures, limits of sensitivity, and coefficients of variation were similar to those described elsewhere (13). Prolactin was assayed as described previously (7). All prolactin values are expressed in terms of prolactin standard VLS αl (NIAMDD) with a biopotency of 28 IU/mg in the crop sac assay.

Analysis of data. All observations from all experiments were used for analysis by the method of least squares. Use of a multiple regression program from the University of Illinois SSUPAC Statistical Services package permitted adjustment of treatment means for contribution by animals and by steroids prior to performing the desired statistical comparisons between means. In this analysis we compared the main effects of steroid treatment (E vs P vs control before, during, and after ES) and the effects of each specific steroid regimen, e.g., two E implants for 24 hr vs two E implants for 30 hr.

Results. *Effects on LH release.* Figure 1 illustrates a representative pattern of changes in concentrations of LH and prolactin in the serum before, during, and after ES. Concentrations of both hormones were relatively stable before stimulation, rose significantly during ES, and fell after ES. This basic pattern was observed under all treatment regimens.

The effects of steroid treatment on mean LH concentration before, during, and after ES are illustrated in Fig. 2. Figure 2A exhibits the mean LH values for the control, combined E, and combined E plus P reg-

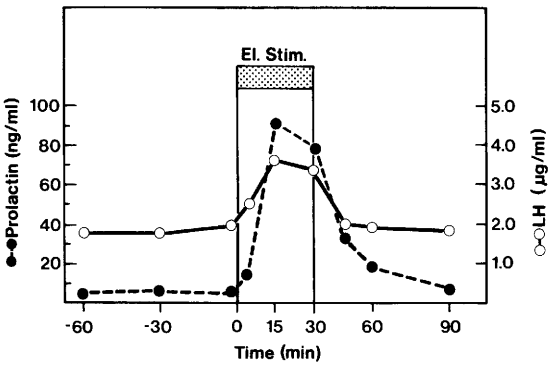

FIG. 1. Representative concentrations of prolactin and luteinizing hormone (LH) before, during, and after electrical stimulation of the mediobasal hypothalamus of an unanesthetized ovariectomized rhesus macaque. Two Silastic capsules containing estradiol had been implanted 24 hr before onset of stimulation. The sample at −1 min was taken immediately before onset of electrical stimulation.

imens and Figs. 2B and C show the effects of individual regimens of E and E plus P, respectively. LH concentrations were reduced significantly ($P < 0.01$) below control values before, during, and after ES by the regimen two E implants for 24 hr (2E24) (Fig. 2B). Significant differences ($P < 0.01$) between 2E24 and 2E30 also were evident before, during, and after ES (Fig. 2B). However, there were no significant differences ($P > 0.05$) in mean serum LH concentrations among groups treated with control, 1E30, and 2E30 regimens.

The main effect of progesterone on serum LH levels (adjusted for effect of estrogen and animals) was highly significant ($P < 0.01$) before, during, and after stimulation (Fig. 2A). Furthermore, there was a significant ($P < 0.05$) linear relationship between the duration of progesterone treatment and

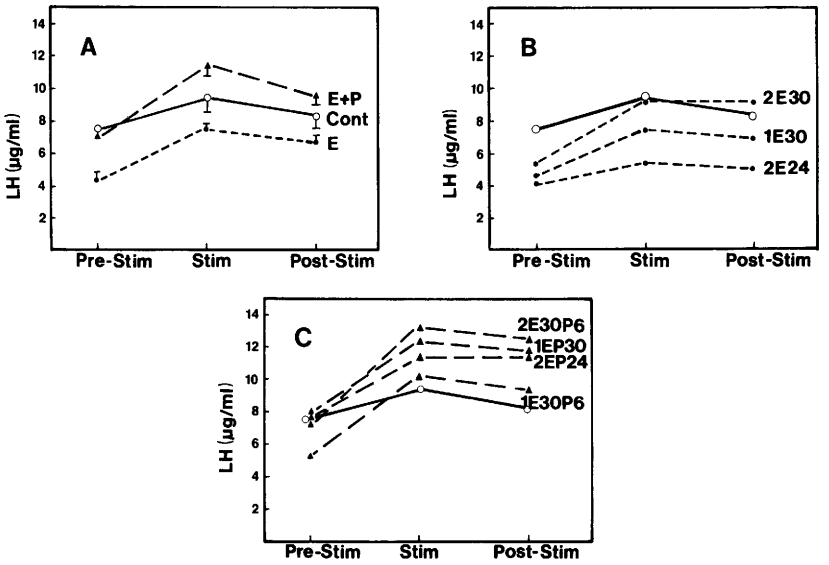

FIG. 2. (A–C) Concentrations of luteinizing hormone (LH) in the serum before, during, and after electrical stimulation of the mediobasal hypothalamus of ovariectomized rhesus macaques which had been treated with Silastic implants containing either no steroid (controls), or estradiol (E) alone, or E and progesterone (P). Panel A exhibits the mean ± SEM concentrations in animals treated with control implants with those treated with several combinations of E and E plus P. Bars indicating SEM of prestimulation means were omitted where values overlapped. Panel B exhibits the effect of three different regimens of E and the control treatments. Panel C exhibits the effects of four different combinations of E and P and the control treatments. The symbols to the right of each line refer to the number and kind of implants and duration of treatment, e.g., 2E30P6 means two implants for 30 hr and one implant for 6 hr immediately before the onset of sampling. The solid line with open circles represents the control in all panels. The graphed values have not been adjusted for differences between animals. In all panels each point represents the mean of data collected for each of the three sampling times before, during, and after stimulation for all animals subjected to a given treatment, e.g., each of the points for a control value represents the mean of 33 observations.

serum LH concentrations during and after ES (Fig. 3) i.e., the effect of P was greatest at 6 hr then declined at 24 and 30 hr. ES significantly elevated serum LH concentrations under all regimens. There was neither a significant interaction between steroid regimens and ES nor between P and E, thus the effects of the steroids appear to be additive.

Effects on prolactin release. The effect of steroid treatments on mean prolactin concentrations before, during, and after ES are shown in Fig. 4. Neither E nor P had any significant ($P > 0.05$) main effect on mean prolactin concentrations before, during, or after ES (Fig. 4A). However, mean prolactin concentrations in sera of animals treated with 2E24 were suppressed significantly ($P < 0.01$) below those treated with 2E30 (Fig. 4B). There were no significant differences between groups in prolactin concentrations of animals treated with different regimens of E plus P (Fig. 4C).

During ES, dose of E significantly affected the slope of the prolactin response with time: the slope was more positive as dose increased from zero to one to two implants for 30 hr (Fig. 5); i.e., the greater the dose the greater the slope. After ES the *mean* concentration of prolactin increased significantly ($P < 0.01$) with dose of E within time (0E at 30 hr (control) vs 1E at 30 hr vs 2E at 30 hr) (Fig. 5). Also, there was a significant linear effect of dose on the rate of change (decline) in serum prolactin after ES. As the dose of E increased (0E at 30 hr vs 1E at 30 hr vs 2E at 30 hr) the slope of the change after ES became less negative.

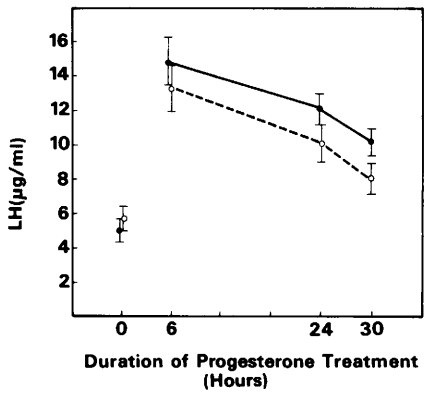

FIG. 3. Effect of duration of progesterone treatment on mean (±SEM) LH concentrations during (●) and after (○) electrical stimulation of the mediobasal hypothalamus. The indicated values were derived from the raw data by the method of least squares and thus have been adjusted for effects of animal and estrogen. The values for 0 hr of treatment represent values for animals not treated with progesterone.

Discussion. These data suggest that exposure of the hypothalamic-hypophyseal system of the rhesus macaque to quasi-physiological concentrations of either E or E plus P for relatively short time periods can selectively alter both basal LH release and LH release in response to ES of the MBH. The effects of the steroids appear to be both time and dose-dependent.

The effect of E on LH concentrations varied with regimen. The circulating concentration of E produced by one implant (116 pg/ml) probably was simply too low to exert a significant effect on LH concentrations. The significant depression of serum LH after 24 hr of treatment with two implants versus no significant effect after 30 hr with two E implants reflected the well-known biphasic effect of this concentration of E on LH secretion—transient depression followed by elevation and eventually an LH surge. Thus at 24 hr LH levels were suppressed temporarily by E but by 30 hr had returned to baseline as the LH surge developed. The depressing effect of treatment with two E implants for 24 hr on the LH release induced by ES was consistent with our earlier results (12).

LH levels were higher in E-plus-P than in E-treated animals prior to, during, and after ES. Thus, P not only prevented inhibition by E but, in combination with E, facilitated both basal and electrically induced LH release. These findings are consistent with the hypothesis that the transient periovulatory rise of P may facilitate LH release in the primate (6). Although the precise effect of P on the periovulatory LH surge is not known, it may alter both the timing of maximal gonadotropin release induced by E and the amount of LH released (5).

The specific site at which P acts to affect LH release in primates is unknown, but it may be the brain, pituitary, or both. Terasawa and Noonan (14) reported that pentobarbital blocks P-induced LH release

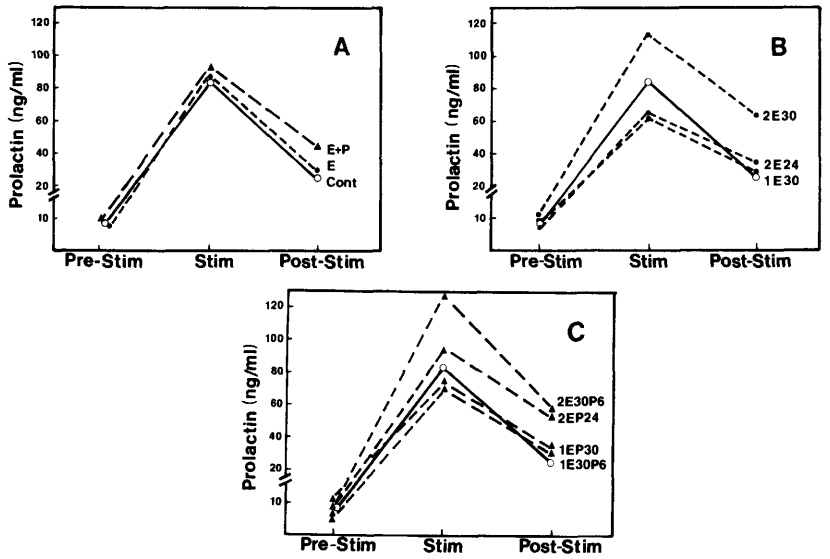

FIG. 4. (A–C) Concentrations of prolactin in the serum before, during, and after electrical stimulation of the mediobasal hypothalamus of ovariectomized rhesus macaques which had been treated with Silastic implants containing either no steroid (controls), or estradiol (E) alone, or E and progesterone (P). Panel A exhibits the mean concentrations in animals treated with control implants with those treated with several combinations of E or E plus P. Panel B exhibits the effect of three different regimens of E and the control treatments. Panel C exhibits the effects of four different combinations of E and P and the control treatments. See legend to Fig. 2 for other details.

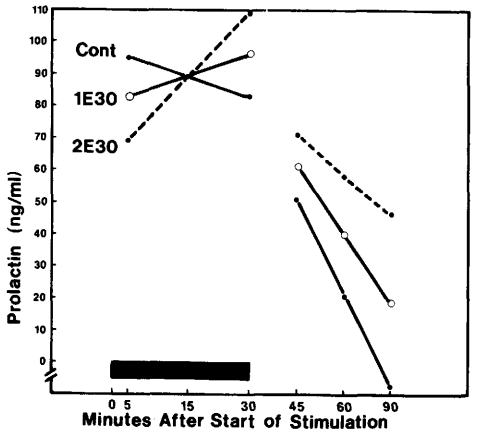

FIG. 5. Mean changes of estimated concentrations of prolactin in the serum of ovariectomized rhesus macaques during and after electrical stimulation of the mediobasal hypothalamus. The indicated values were derived from the raw data by the method of least squares and thus have been adjusted for differences between animals. The black bar between 0 and 30 indicates duration of electrical stimulation. The notations at the left indicate number and kind of implant and duration of treatment prior to onset of sampling.

in ovariectomized macaques and suggested that the brain is the site of action of P. Detailed studies in rats suggest that the facilitative effects of P on LH release in that species occur in both the pituitary (15, 16) and hypothalamus (17). The negative feedback effects of P appear to be predominantly on the anterior pituitary (16, 18, 19), but also may be on the preoptic area (20).

Neither E nor P altered, from control values, the mean concentrations of prolactin before ES. However, E affected, in a dose-related manner, patterns of prolactin release during ES. This effect of E also was reflected after stimulation both in mean concentrations of prolactin and in the rate of fall of prolactin with time. This effect of E is difficult to interpret, but it suggests that E affects, in a dose related manner, the "reserve" of prolactin. In the absence of E, the response to ES peaked quickly (at 5 min). In the presence of E the response peaked more slowly, but at a higher level, and then declined more slowly (Fig. 5).

Previous studies by Quadri *et al.* (11) showed that treatment of ovariectomized monkeys with E for 72 hr significantly increased the release of prolactin in response to ES of either the MBH or the rostral hypothalamus. The importance of duration of exposure to E is reflected in the significant effect of time of exposure (2E at 24 hr vs 2E at 30 hr) on basal prolactin concentrations seen in this study. The time dependence of the estrogen effects on prolactin secretion also has been observed by other investigators. *In vivo* treatment of rats with E causes a measurable increase in RNA for preprolactin within 24 hr (21), but maximal effects occur only after 7 days of treatment (22).

The lack of an effect of P on prolactin secretion was consistent with other observations in rhesus macaques, i.e., no marked change in prolactin levels during the menstrual cycle. However, P has variable but demonstrable effects on prolactin secretion in other species. In sheep, P either increases (23) or does not alter (21) prolactin secretion *in vivo*. In contrast, an inhibitory effect of P *in vivo* occurs in bulls (24). Also, P antagonizes the stimulatory action of E on prolactin secretion by rat and sheep pituitary cells *in vitro* (25, 26).

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