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In Vivo and In Vitro Studies on the Differential Role of Luteinizing Hormone and Follicle-Stimulating Hormone in Regulating Follicular Function in the Bonnet Monkey (*Macaca radiata*) Using Specific Gonadotropin Antibodies[1]

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ABSTRACT

Although a distinct need for FSH in the regulation of follicular maturation in the primate is well recognized, it is not clear how FSH controls the functionality of different cellular compartments of the follicle. It is also not evident whether there is a requirement for LH in follicular maturation in the primate. In the first part of the present study, female bonnet monkeys were administered a well-characterized ovine (o) LH antiserum to neutralize endogenous monkey LH for different periods during the follicular phase, and the effect on the overall follicular maturation process was assessed by analyzing serum estrogen (E) and progesterone (P) profiles. Neither continuous LH deprivation from Day 8 of the cycle nor deprivation of LH on any one day between Days 6 and 10 had a significant effect on serum E and P profiles and the follicular maturation process. The period for which the antiserum was effective was dependent upon the dose injected; 1 ml of the antiserum given on Day 8 blocked ovulation but not follicular maturation.

To assess the effect of deprivation of LH/FSH at the cellular level, animals were deprived in vivo of LH (on Days 8 and 9 of the cycle) or of FSH (on Day 6 of the cycle) by injection of highly characterized hCG and ovine (o) FSH antisera, respectively; the in vitro responsiveness of granulosa and thecal cells isolated on Day 10 from these animals was then determined. The in vitro responsiveness of granulosa but not thecal cells to added hCG (2 ng/ml) was inhibited (by 53%) by neutralizing LH in vivo; granulosa cell responsiveness to added FSH, however, remained unaltered. In contrast, neither granulosa nor thecal cells isolated on Day 10 of the cycle from animals injected with an FSH antibody on Day 6 responded in vitro to added FSH or LH. These data suggest that 1) follicular maturation in the nonhuman primate can proceed even when endogenous LH support is withdrawn from Day 6 onward; 2) while granulosa cells are sensitive to deprivation of LH for the short term, thecal cell functionality appears not to be dependent on LH from Day 6 of cycle onward; and 3) as FSH controls granulosa cell function as well as thecal cell function (perhaps via paracrine factors), this hormone qualifies as the prime regulator of follicular maturation in the primate.

INTRODUCTION

The process of follicular maturation in primates can be broadly classified into recruitment, selection, and dominance, the last phase being accompanied by a burst in functional maturation leading to the formation of an ovulable follicle [1]. At the cellular level, this involves proliferation and differentiation of the granulosa and thecal cells and their ability to respond to both gonadotropins. FSH in turn stimulates the induction of growth factors in addition to aromatase responsible for E synthesis in granulosa cells [2, 3].

The adult female bonnet monkey (*Macaca radiata*), which has been shown to be an excellent human surrogate model, exhibits a follicular phase of 11–12 days with an E surge occurring between Days 8 and 10 and ovulation between Days 11 and 12 of the cycle [4]. Using characterized FSH antibody to block FSH action, we have previously shown that in the female bonnet monkey, FSH is required only until Day 8 of the cycle, the follicle becoming highly sensitive to the lack of FSH between Days 5 and 7 of the cycle [4]. Neutralization of FSH on Day 8 or beyond had a very negligible effect on the E surge, an index used for determining completion of follicular maturation. While studies from other laboratories have essentially confirmed the need for FSH [5–8], no information is available on exactly how FSH regulates the function of different cellular compartments in the follicle.

Information on the precise requirement for LH in follicular growth and development is lacking; in particular, since the need for FSH is restricted to the first 7–8 days of the cycle [4], we wished to determine whether LH assumes a dominant role from Day 8 on. Schenken et al. [9] were able to induce growth of multiple follicles in cynomolgus monkeys by administering only "pure FSH" and thus questioned the relative importance of exogenous LH administration during the follicular phase. In light of the success we have had in the past using specific FSH antibodies to study the role of FSH in regulation of follicular maturation in monkeys [4], rodents [10–13], and rabbits [14], in the present study we used specific LH antibodies to delineate the role of LH in regulating the overall follicular maturation process in cycling monkeys. In addition, we have attempted to understand how FSH and LH regulate the functionality of different cellular compartments of the follicle by studying how in vivo deprivation of LH or FSH on specific day(s) of the cycle affected the ability of isolated granulosa and

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thecal cells to respond to these gonadotropins in vitro. This has enabled us to establish a paracrine role for granulosa cells in regulating thecal cell functionality.

MATERIALS AND METHODS

Hormones and Chemicals

Human (h) CG (CR127; 14 900 IU/mg) and hLH (NIH AFP 4745B) were kindly provided by Dr. G. Bialy, NICHD, Bethesda, MD. Ovine (o) FSH (1 ng oFSH corresponds to 1.46 ng hFSH, NIH AFP 4822 B) and LH (1 ng is equivalent to 0.13 ng hLH AFP 4745B) were purified in our laboratory. DMEM was from Gibco Labs. (New York, NY); collagenase, HEPES, and $NaHCO_3$ were from Sigma Chemical Co. (St. Louis, MO). Tritiated progesterone (P; 88 Ci/mmol), estradiol (E_2; 90 Ci/mmol), testosterone (T; 97 Ci/mmol), and ^{125}I Na were purchased from Amersham (UK).

General Methodology

Healthy adult regularly cycling female bonnet monkeys (*M. radiata*), 8–12 yr of age, were used. The details of animal husbandry, monitoring of the cycle, and procedure for collection of blood samples have been previously described [15]. All the animal experiments were cleared by the institutional ethical committee for use of laboratory animals for biomedical research. The day of onset of menstrual bleeding was considered Day 1 of the cycle. Antiserum was administered, when needed, i.v. at 0930 h. Blood samples were collected at appropriate times from unanesthetized monkeys by venipuncture using vacutainer tubes (Beckton and Dickinson, Rutherford, NJ) as described earlier [4].

Antisera

Antiserum against oFSH was raised in male bonnet monkeys as described previously [4]. The oFSH antiserum used in the current study was from the same pool as used previously in our laboratory, and the methods for characterization of the antiserum for capacity, affinity, cross-reactivity, and minimum effective dose (MED) were described in that study [4].

Antibodies capable of blocking endogenous monkey LH were produced by immunizing donor male bonnet monkeys with oLH and adult goats with hCG. The methods used for immunization are well-standardized laboratory procedures that have been previously described in detail [16]. Methods used for determining the characteristics of the antisera—such as cross-reactive antibody titer, specificity, bioneutralization of monkey LH, and MED—were essentially the same for both batches of antisera (oLH and hCG) and are described in brief below. Since the goat antiserum to hCG was from a heterologous species, it was precipitated with 15% Na_2SO_4 and then extensively dialyzed against water to obtain the enriched IgG fraction that was used.

To determine the ability of the antisera to bind monkey LH, the efficacy of the LH/hCG antibody to prevent monkey pituitary extract (MPE; used as a source of monkey LH) from inhibiting ^{125}I-hLH binding to sheep luteal membrane was examined via an LH radioreceptor assay (RRA) described earlier [17]. An aliquot of MPE (equivalent to 1.9 ng hLH) was incubated at 4°C overnight with different concentrations of oLH/hCG antisera to form an antigen-antibody complex; then the ability of the complex to inhibit binding of ^{125}I-hLH to LH receptor was checked in an appropriate RRA.

Determination of MED of the LH/hCG Antisera

Two types of experiments were conducted to determine the MED of the LH/hCG antisera. In the first, varying doses of the LH/hCG antisera (0.05, 0.2, and 1.0 ml for LH; 0.025, 0.05, and 0.1 ml for hCG) were injected on Day 8 of the cycle to a group of regularly cycling monkeys. Then the time taken for clearance from circulation of the antibody cross-reacting with primate LH was assessed by the rate of disappearance of free antibody capable of binding ^{125}I-hLH.

To 100 μl of the serum collected at Time 0 of antiserum administration and at subsequent time points, ^{125}I-hLH (~100 000 cpm) was added and the volume was made up to 0.5 ml with RIA buffer. This was incubated at room temperature for 12–14 h. The bound and free antibody were separated by addition of polyethylene glycol (PEG 6000; 12.5% final concentration), henceforth called the PEG method. Five hundred microliters of 25% PEG was added to the incubation mixture, vortexed, and kept at 4°C for 30 min; this was followed by spinning at 1600 × *g* for 25 min. The supernatant was discarded; the pellet was suspended in 1 ml buffer containing 12.5% PEG and subjected to an additional wash step. The final pellet was monitored for radioactivity in a multigamma counter. The presence of free antibody during the various time points after injection of the antiserum was calculated.

The second model system consisted of determining the ability of LH/hCG antibody to block the occurrence of the nocturnal T surge in adult male bonnet monkeys. Before monkeys were used in the experiment, their serum T levels at 2200 h of Days −2 and −1 were determined. This was essentially to establish the normal occurrence of increased serum T levels at nocturnal times in these monkeys. Different doses of LH/hCG antibody were administered to male monkeys at 0930 h; serum samples collected at 2200 h of the same day and the subsequent two days were analyzed for T level.

Granulosa and Thecal Cell Isolation and Incubation Protocol

Laparotomy was performed under ketamine anesthesia. The dominant follicle was aspirated by means of a 24-gauge needle mounted on a tuberculin syringe. The antrum was

gently flushed twice to remove the loosely held granulosa cells with DMEM containing 25 mM HEPES, 0.37% $NaHCO_3$, and 0.1% BSA, pH 7.4 [18]. The thecal tissue was excised and placed in the medium. The follicular aspirate was centrifuged, the fluid was separated, and the cells were suspended in DMEM. The medium obtained from flushing the follicle was also centrifuged to collect residual granulosa cells, and the cells thus obtained were all pooled and used. The thecal tissue was extensively washed to remove any contaminating granulosa cells and then was subjected to collagenase digestion essentially according to Stouffer et al. [19], with slight modifications to obtain single cells. In brief, this procedure consisted of cutting the thecal tissue into small pieces, suspending them in DMEM containing 2% BSA and 0.2% collagenase, and incubating at 37°C in a shaking water bath for 15 min. Then 2 ml of DMEM containing 2% BSA was added, and the single cells were released by repeatedly aspirating and releasing the suspension with a pipette for about a minute to free the dispersed cells. After the debris was allowed to settle, the supernatant containing the cells was aspirated and subjected to a wash step with the medium. The debris was further digested twice; the supernatants from different digests were pooled, and the cells collected by centrifugation were washed three times to remove excess collagenase before the final cell pellet was suspended in a fixed volume of medium.

The granulosa and thecal cells in the DMEM medium were preincubated at 37°C for 30 min in a shaking water bath followed by washing, and the cells were suspended in a known volume of medium. Cells were counted in a hemocytometer; 5×10^4 viable cells per tube (as determined by trypan blue exclusion) were incubated in triplicate in the presence and absence of the gonadotropins (both hCG and oFSH) in a total volume of 0.5 ml (in 22 × 75-mm glass tubes) for 3 h at 37°C in a shaking water bath at 60 oscillations per minute. After the incubation, the tubes were kept frozen at −20°C until assayed for the appropriate steroid hormone.

Hormone Assays

E_2, P, and T assays were carried out as described elsewhere [16]. The serum samples were ether extracted twice and suspended in gelatin PBS (GPBS). The in vitro incubates were assayed for P without any processing. The P and T antisera used were kind gifts of Dr. Chandana Das, AIIMS, New Delhi, and Dr. Usha Joshi, IRR, Bombay, respectively. Characteristics of the P and T antisera used have been described by Ravindranath et al. [4] and Moudgal et al. [20], respectively. The antibody to E_2 raised in our laboratory cross-reacted 3.7% and 1.1% with estrone and estriol, respectively; for P and T, the cross-reactivity was less than 0.1%.

Statistics

Individual experiments were carried out using a minimum of 3–4 animals per group, and the data presented are means and SEM of replicate experiments. In the in vitro incubation study, the response to each level of hormone addition was determined in triplicate. Treatment differences were analyzed by Student's *t*-test.

RESULTS

Characteristics of the FSH Antiserum

The FSH antibody used in the current study was from a pool of monkey antiserum to oFSH whose characteristics were previously described in detail by Ravindranath et al. [4]. In brief, the affinity and the capacity of this oFSH antiserum to bind hFSH were 0.3×10^{10} M^{-1} and 43.3 μg/ml, respectively. This antibody cross-reacted < 10% with hLH (used as a representative of primate LH). Earlier studies in adult female cycling monkeys had indicated that the MED of this antiserum required to neutralize endogenous FSH action over a 24-h period was 25 μl [4].

Characteristics of the Antisera Used to Neutralize LH

The binding affinity and capacity of the monkey antiserum to oLH, as determined by Scatchard analysis using hLH as the radioligand, were 0.12×10^8 M^{-1} and 33 μg/ml hLH, respectively. The corresponding values for the goat antibody to hCG were 1.3×10^9 M^{-1} and 405 μg/ml hLH, respectively. The cross-reactivity of both these antibodies with hFSH was < 8%. In the ^{125}I-hLH receptor assay, the ability of the monkey pituitary extract (containing 1.9 ng hLH equivalent) to inhibit labeled ligand binding was blocked on pre-incubation with either the oLH or the hCG antibody, and this was a function of antibody concentration. Excess antibody showed a nonspecific effect due to direct interference of the antibody with ligand binding; but when the dose of the antibody became optimal (achieved by adding decreasing amounts of antibody) there was 100% binding by the radiolabeled ligand, indicating that monkey LH was completely bound by the antibody (equivalence zone). In the case of the hCG and oLH antibodies, this occurred with 28 and 230 ng IgG, respectively.

Determination of MED of the Antibody

Both the LH and hCG antisera were tested in cycling monkeys for their clearance rate; Figure 1 depicts the results of a typical experiment with monkey antiserum to oLH performed in a group of female bonnets on Day 8 of the cycle. Although after administration of 50 μl of antiserum no "free" antibody could be detected by the ^{125}I-hLH binding test even at 2 h, injection of 200 μl antiserum revealed the presence of excess "free" antibody capable of binding ^{125}I-hLH in substantial amounts (60% and 37%) at 6 and 24 h, respectively. After administration of 1 ml of antiserum, appreciable binding (49%) to ^{125}I-hLH could be detected even 6 days after the injection. These findings indicate that the duration for which the antibody is effective is a function

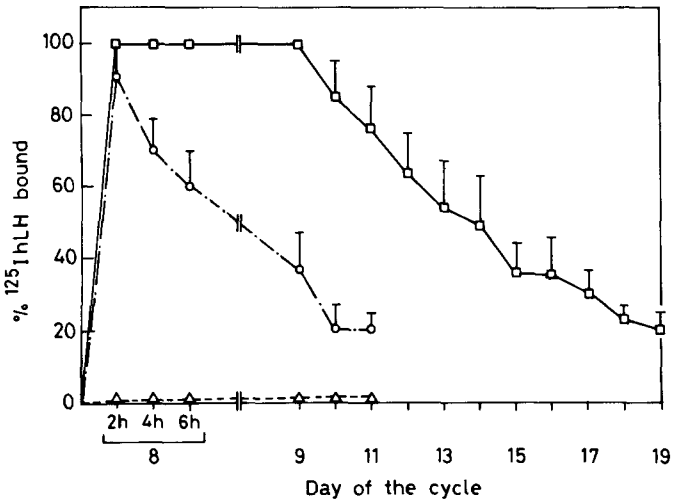

FIG. 1. Disappearance of antibody cross-reacting with primate LH as a function of time after injection of different volumes of monkey antiserum to oLH on Day 8 of the cycle in female bonnet monkeys. Mean ± SEM; n = 3. Open triangles, 50 μl; open circles, 200 μl; open squares, 1 ml

of the dose of antibody injected (Fig. 1) and this is reflected by the "free" antibody titer. The MED was set at 200 μl/day, as at this dose a substantial amount of free antibody could still be detected 24 h after injection. The MED of the hCG antibody as determined in this assay was 25 μl (data not shown).

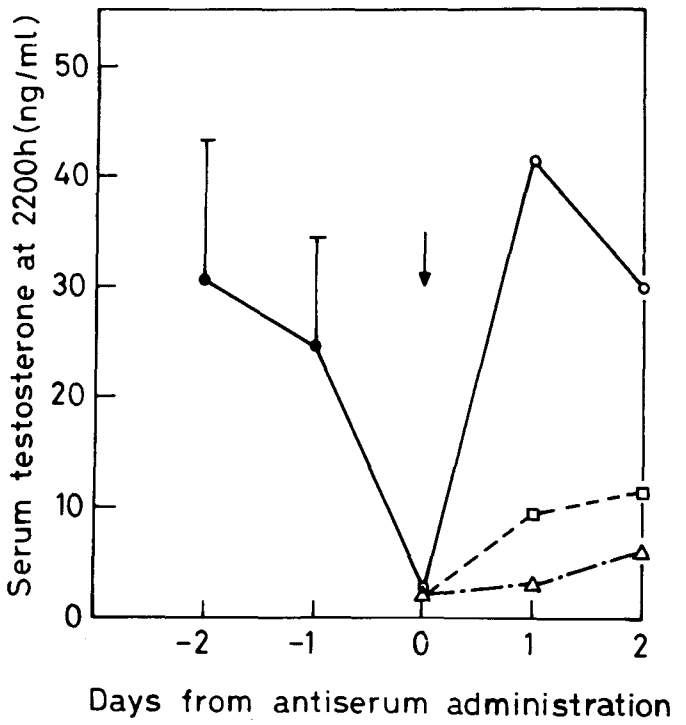

FIG. 2. Effect of administration of different doses of hCG antiserum (arrow) at 0930 h on serum T level at 2200 h in male bonnet monkeys. Serum T levels at 2200 h of Days −2, −1, 0, 1, and 2 are provided. Each of the values is a mean of values for 2 monkeys except during pretreatment, when all 6 monkeys were considered for SEM determination. Solid circles, pretreatment; open circles, 25 μl hCG antibody; open squares, 50 μl hCG antibody; open triangles, 100 μl hCG antibody

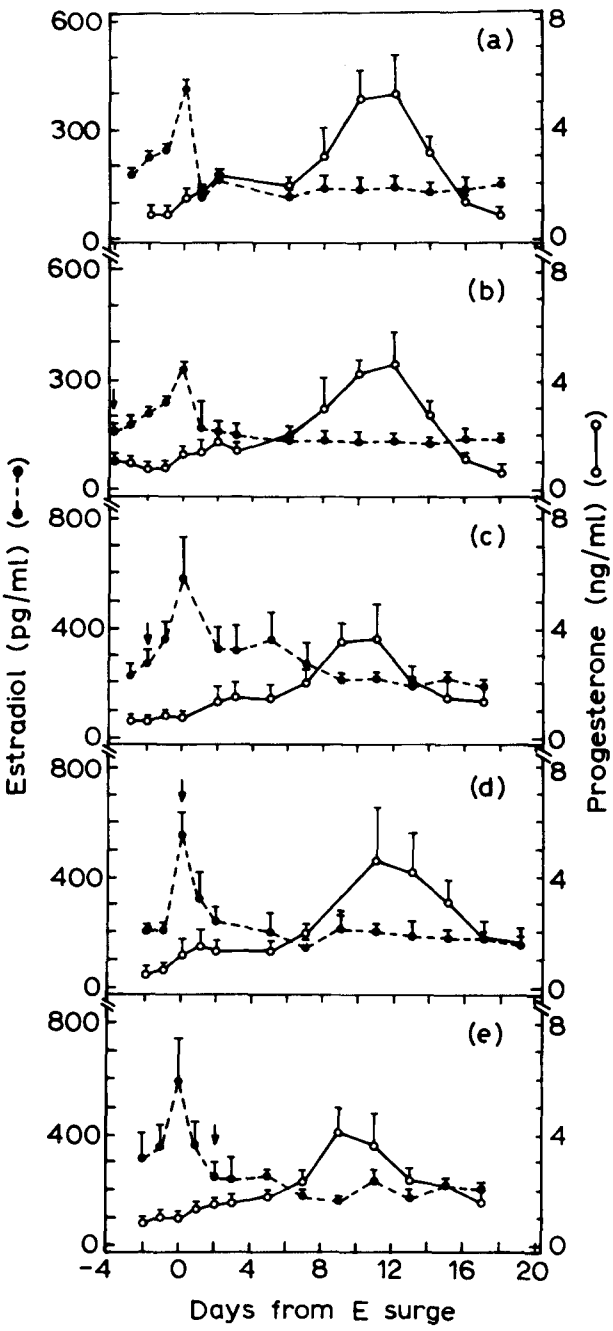

FIG. 3. Effect of injecting (arrow) 200 μl of oLH antiserum on different days of the cycle on serum E and P profiles during the cycle. Steroid level (a) in controls and (b-e) after LH antiserum administration on Days −4, −2, 0, and 2 of E surge, respectively. Values represent mean ± SEM. Number of monkeys used in each group was 3 or 4 (see Table 1).

Figure 2 depicts the results of a typical experiment conducted with different doses of the hCG antiserum (25, 50, and 100 μl) to determine the MED required to block increment in the serum T level at 2200 h (beginning of nocturnal T surge). A dose of 25 μl of hCG antibody was adequate to block the elevation in the serum T level seen at

TABLE 1. Effect of LH deprivation during different periods of follicular phase on cycle length and progesterone production in the luteal phase.

Day of LH antiserum administration*	n	Cycle length (days)	Total progesterone secreted during the luteal phase (Area under the curve cm^2)#	% Change over the control
Control	3	28.6 ± 0.88	45.04	—
−4	4	28.0 ± 0.81	40.39	−10
−2	4	27.5 ± 1.70	32.30	−28
0	3	31.3 ± 1.76	41.45	−8
+2	4	27.0 ± 2.08	37.18	−17

*Day 0 represents day of estrogen surge (normally occurs between Days 8 and 10 of the cycle). Days of LH antibody administration have been computed with reference to the day of E surge. In all cases the LH antibody was injected at 0930 h. − = before E surge. + = after E surge.

#Progesterone from Day 2 following E surge to the end of the cycle was considered to compute the area under the curve.

2200 h, and this effect lasted for only a day (Fig. 2). Administration of 50 or 100 μl of hCG antibody resulted not only in blockade of the nocturnal serum T surge on the day of treatment but also in substantial inhibition of the nocturnal serum T increment on the next two days (Fig. 2). The MED of LH antibody in this assay was 200 μl/monkey/day (data not provided). The rationale for using the adult male monkey model to determine the MED of the two antisera is as follows. 1) It is known that the LH levels of adult male and female bonnet monkeys (during the follicular and luteal phase) are essentially similar [17]; 2) it is easier to procure adult males in numbers than to procure cycling females that are all on a particular day of the cycle; and 3) it has been demonstrated that reduction in serum T levels is a sensitive indicator of endogenous LH availability [21].

Effect on Cyclic Ovarian Function of Administering LH Antibody on Different Days of the Follicular Phase

Normally cycling female monkeys (n = 3) receiving 1 ml of oLH antibody on Day 8 of the cycle showed, on laparotomy on Day 14, a single well-developed follicle and no signs of recent ovulation (lack of follicle with stigmata or early CL). It is interesting to note that even injection of a relatively large amount of LH antiserum on the morning of Day 8 had no effect on the occurrence of the E surge, which appeared between Days 8 and 10 of the cycle (data not shown). Administration of 1 ml LH antiserum on Day 8 was considered (on the basis of data included in Fig. 1) sufficient to block LH at least until Day 14. The block in ovulation (verified at laparotomy) is presumed to have been due to neutralization of the preovulatory LH surge. The dominant nonovulated follicle present on Day 14 of the cycle must, therefore, represent an atretic follicle, as the granulosa cells isolated from it in short-term incubation studies did not respond to added (20 ng/tube) hCG (P secreted: control, 1.79 ± 0.20 vs. experimental, 1.8 ± 0.13 ng/ml/3 h/10^5 cells). This experiment also provided the first clue that a block in LH action from Day 8 onward does not lead to arrest in follicular maturation per se.

Administration of MED of the oLH antibody (200 μl/monkey) on different days of mid-follicular phase had a negligible effect on the cycle length or the steroid profile during the cycle. The results are normalized to the day of the E surge for the purpose of comparison (Fig. 3 and Table 1). Deprivation of LH 2 days before or 2 days after the E surge resulted in a marginal decrease (28% and 17%, respectively) in production of luteal P. Administration of LH antibody 4 days before or on the day of the E surge, however, resulted in virtually no change in occurrence of the E surge or in luteal P secretion. Cycle length in the treated animals varied between 27 and 31 days, which was not significantly different from the cycle length for controls (Table 1).

Responsiveness to Added Gonadotropins in Vitro of Granulosa and Thecal Cells from Animals Deprived of FSH on Day 6 or LH on Days 8 and 9

For a closer look at the effect that LH and FSH neutralization in vivo produces on the functionality of follicular cell types, short-term incubations were performed with granulosa and thecal cells isolated on Day 10 of the cycle in the presence or absence of added gonadotropins. Our earlier study [4] showed that the follicular maturation process in the bonnet monkey is highly sensitive to FSH deprivation between Days 5 and 7 of the cycle; consequently in the current study, we investigated the effect of injection of FSH antibody on Day 6 of the cycle on granulosa and thecal cell responsiveness in vitro on Day 10 of the cycle.

Administration of hCG antibody (25 μl) on Days 8 and 9 of the cycle significantly inhibited the responsiveness of the granulosa cells to added hCG (2 ng/ml). However, these cells' responsiveness to FSH (20 ng/ml) to secrete P remained unaltered, indicating that deprivation of LH had affected only the homologous responsiveness (Fig. 4). FSH deprivation on Day 6 of the cycle, however, inhibited the responsiveness of granulosa cells in vitro to both added hCG and FSH (Fig. 4). The doses of gonadotropin added were chosen from a pilot experiment in which the optimal

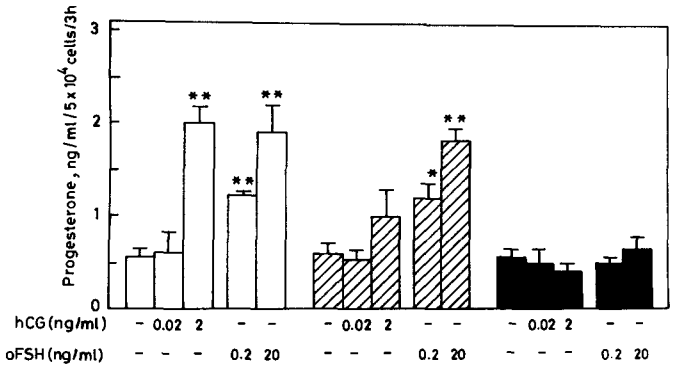

FIG. 4. Steroidogenic response to added gonadotropins in vitro of granulosa cells isolated on Day 10. Cells isolated from: controls, open bars; animals deprived of LH on Days 8 and 9, crosshatched bars; and animals deprived of FSH on Day 6, solid bars. Values represent mean ± SEM of 9 samples. For each hormone level, incubation was carried out in triplicate (5×10^4) cells per tube) for 3 h at 37°C, and each group comprised 3 animals. For procedural details refer to text. *$p < 0.05$, **$p < 0.005$ (Student's t-test) compared to incubated control.

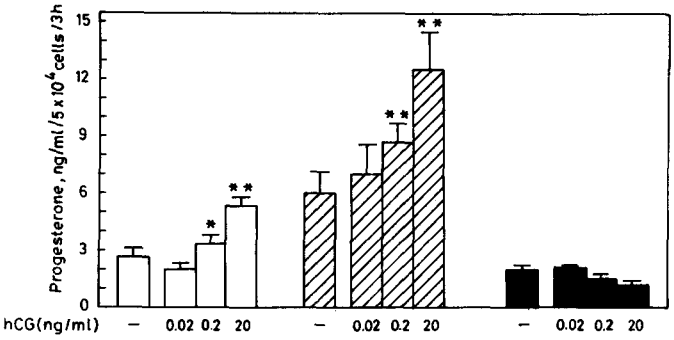

FIG. 5. Steroidogenic response in vitro to added hCG of thecal cells isolated from Day 10 of the cycle from control and experimental monkeys receiving different treatments in vivo. Controls, open bars; cells from animals treated with hCG antibodies on Days 8 and 9, crosshatched bars; cells from animals treated with FSH antiserum on Day 6, solid bars. P secreted was quantified by RIA. Values given are mean ± SEM of 9 samples. For each hormone level, incubation was carried out in triplicate (5×10^4) cells per tube) and each group consisted of 3 monkeys. *$p < 0.05$, **$p < 0.005$ (Student's t-test) compared to incubated control.

dose of hCG or FSH needed to maximally stimulate P production was determined.

In contrast to findings for the granulosa cells, the responsiveness of the thecal cells, which possess only LH/CG receptor, was not affected by LH deprivation on Days 8 and 9 of the cycle (Fig. 5). There was a dose-dependent increase in P production by the thecal cells to added hCG (0.02, 0.2, and 20 ng/ml); P output in fact was higher than in the controls. It appeared from this observation that the thecal cell responsiveness in vitro, which is also a measure of the presence of a normal quota of LH receptors, is not influenced by the absence or presence of endogenous LH support. On the other hand, FSH deprivation on Day 6 led to a total block in hCG responsiveness of thecal cells on Day 10; this was particularly interesting since endogenous LH levels have been shown to remain unaltered after active immunization against oFSH in monkeys [20].

DISCUSSION

Earlier studies from our laboratory have unequivocally demonstrated that there is a critical need for FSH in promoting follicular maturation until Day 8 of the cycle, the follicle becoming extremely sensitive to deprivation of FSH between Days 5 and 7 of the cycle [4]. Assuming that follicular maturation beyond Day 8 of the cycle and ovulation (occurring on Day 11 or 12 of cycle) could be regulated by LH, an attempt was made in the current study to deprive cycling adult female bonnet monkeys of LH support by administering characterized LH antibody on different days starting from the mid-follicular phase. The batches of LH/hCG antisera used were specific to LH and were capable of bioneutralizing circulatory monkey LH effectively. Their cross-reactivity with FSH was less than 8%. The affinities of both the antisera to bind LH were also of a high order. A variety of in vivo and in vitro methods have been used to determine the efficacy (to neutralize monkey LH) and MED of the LH and hCG antisera. On the basis of affinity, capacity, bioneutralizing ability, and clearance of antibody from circulation, the MED of the LH antibody was set 8 times higher (200 μl) than that of the hCG antibody (25 μl).

After administration of 1 ml of LH antiserum on Day 8, the presence of a mature dominant follicle rather than an ovulated follicle/CL on Day 14 of the cycle indicated 1) that continuous neutralization of circulating LH had no effect on follicular maturation per se and 2) that since ovulation had not occurred, the quantity of antibody was adequate to block even the preovulatory LH surge. This treatment also had no effect on the E surge, indicating that E synthesis beyond Day 8 is independent of LH support. Confirmation of the lack of effect on E production was obtained by administering MED of LH antiserum (200 μl/monkey) between Days −4 and 0 (the day of E surge). This finding was in marked contrast to our earlier observations on FSH antiserum showing that FSH deprivation up to Day −1, but not beyond, results in a drastic reduction in E secretion [4]. The present study has further shown that blocking LH action for a 24-h period during the mid-follicular and periovulatory phase (Day −4 to Day 2 of E surge) has no effect on ovulation, luteal P production, or cycle length. The fact that MED of LH antibody (200 μl) did not block ovulation suggests that the dose administered was not adequate to block the LH surge that precedes ovulation. This set of results, as far as we are aware, provides the first direct proof that LH is not needed for the promotion of follicular maturation per se in the nonhuman primate. In their study on active immunization of female rhesus monkeys against oLH ß-subunit, Thau et al. [22] noted the effect of this treatment on the occurrence of a short luteal phase but failed to comment on its lack of effect on follicular maturation.

Schenken et al. [9] demonstrated that administration of a "pure" FSH preparation alone (without exogenous LH) to normally cycling cynomolgus monkeys could induce multiple follicles to develop, thus placing into question the relative importance of LH for follicular maturation. However, in this model system the role of endogenous LH could not be discounted. Edelstein et al. [23] observed in women treated with GnRH agonist that stimulation with human menopausal gonadotropin or oFSH resulted in similar mean peak E levels and that the number of oocytes aspirated under both treatments was identical. Schoot et al. [24] were able to induce follicular growth up to the preovulatory stage by administering recombinant human FSH to a woman with congenital gonadotropin deficiency. Despite low E levels due to a lack of LH, this woman, interestingly, exhibited normal follicular growth. These in vivo studies are essentially supportive of our contention that FSH and not LH is the primary regulator of follicular maturation.

The results of the in vitro studies are of particular interest, as these provided insight into the differential sensitivity of the granulosa and thecal cell compartments to specific LH or FSH deprivation. Although both granulosa and thecal cells respond to LH, only the former showed sensitivity to LH deprivation in vivo. The responsiveness of the granulosa cells to FSH remained unaltered under conditions of LH deprivation in vivo, clearly indicating that the effect is confined to homologous response only. E synthesis has been shown to occur in both granulosa and thecal cells [25], and the aromatase enzyme of at least the granulosa cell is known to be stimulated by FSH. E synthesis, however, is dependent upon the availability of androgen produced in the thecal cells. In vitro studies with thecal cells isolated from LH-deprived animals, interestingly, showed that the steroidogenic potential of this cell type is not influenced (as are granulosa cells) by the lack of LH in vivo. This perhaps provides a rational explanation for the observation of no change in serum E levels after LH deprivation in vivo. Blocking FSH action for a single day, interestingly, resulted in the granulosa cells losing responsiveness in vitro to both hCG and FSH. Others have reported a similar loss in responsiveness to both added FSH and LH in vitro in granulosa cells isolated from rabbits pretreated with FSH antiserum in vivo [14]. It has also been demonstrated in the marmoset that FSH augments LH-induced steroidogenesis [26]. These results lend support to the current thinking that FSH could be regulating steroidogenic responsiveness of granulosa cells to LH. The current study has further shown that the in vitro responsiveness of thecal cells to LH is also influenced by FSH deprivation in vivo. Considering that administration of FSH antiserum does not lead to change in LH levels [17] and that thecal cells do not have FSH receptors, the block in thecal cell responsiveness to LH in vitro can only be attributed to regulation by FSH of thecal cell function via granulosa cell-secreted paracrine factors. The recent demonstration of Smyth et al. [27] that androgen production of rat thecal cells is modulated by FSH-stimulated granulosa cell paracrine factors is supportive of our results.

Various factors such as insulin-like growth factor-1 (IGF-1), epidermal growth factor, inhibin, activin, tumor necrosis factor alpha, etc., have been reported to be synthesized by primate and rodent granulosa cells [28–33], and at least some of these factors (e.g., inhibin, IGF-1, etc.) have been shown to be increased by FSH in a dose-dependent manner [34]. These factors in turn modulate gonadotropin-stimulated E production by the granulosa cells [31]. Hillier et al. [35] have demonstrated that inhibin and IGF-1 have a paracrine role in modulating LH-stimulated thecal androgen biosynthesis in the human ovary. From the foregoing it is apparent that of the two gonadotropins, the need for FSH in regulating follicular maturation is paramount, as FSH controls—in addition to granulosa cell proliferation—the functionality of both granulosa and thecal cells of the maturing follicle via autocrine/paracrine factors put out by the granulosa cells. The nature of these factors needs to be established.

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