

# The Effect of Estradiol on Hormonally Stimulable Adenylyl Cyclase Activity and on Progesterone Production in Normal and Regressing Corpora Lutea from Control and Human Chorionic Gonadotropin-Treated Pseudopregnant Rabbits\*

SHARON L. DAY AND LUTZ BIRNBAUMER†

*Department of Cell Biology, Baylor College of Medicine, Houston, Texas 77030*

**ABSTRACT.** The effect of administering a luteolytic dose of hCG (100 IU, iv) to 9-day pseudopregnant rabbits that received either no other treatment (controls) or one of two estradiol (E-1.5 or E-15) treatments was studied. E-1.5 and E-15 consisted of 1.5 µg estradiol (E) every 12 h and 15 µg E every 12 h, respectively; these treatments were administered sc in sesame seed oil starting at 2200 h on day 8 and continued through 2200 h on day 11. Animals were bled daily from days 9–12 and sacrificed between 1000–1100 h on day 12, at which time serum and corpora lutea (CL) progesterone content, weight, and adenylyl cyclase activities stimulated by LH (10 µg/ml) and isoproterenol (Iso; 10<sup>-4</sup> M) were determined.

We found that 1) neither low (E-1.5) nor high (E-15) E treatment prevented functional luteolysis (drop in serum progesterone) induced by hCG; 2) E-15 but not E-1.5 prevented structural luteolysis (CL weight loss) induced by hCG; 3) E-15 but not E-1.5 resulted in CL progesterone levels that, after hCG, were indistinguishable from levels in CL of control animals that received either no treatment or E-15 alone; 4) hCG on day 9 alone resulted, by day 12, in the loss of both LH-stimulable and Iso-stimulable CL adenylyl cyclase activities; 5) E-1.5 had no

effect on CL adenylyl cyclase activity and did not protect against hCG; 6) E-15 alone resulted, by day 12, in the appearance of a partially but significantly decreased LH-stimulable activity without affecting either basal or Iso-stimulable activities; and 7) LH and Iso-stimulable activities in CL of E-15-treated rabbits did not differ upon hCG treatment.

We conclude 1) that hCG and LH affect rabbit CL by stimulating adenylyl cyclase, desensitizing adenylyl cyclase, and triggering functional luteolysis; 2) that the luteolytic effect of hCG is direct and independent of E supply; 3) that high doses of E (E-15) but not low doses of E (E-1.5) can protect the CL against the effects of hCG resulting in the loss of hormone-stimulable adenylyl cyclase activities and against the structural luteolysis that follows hCG-induced functional luteolysis; and 4) that progesterone synthesis and secretion in CL may be separately and independently regulated by LH, thus accounting for low serum progesterone in rabbits treated with E-15 plus hCG in spite of the presence of tissue progesterone levels similar to those seen in actively secreting CL of control or E-15 only-treated rabbits. (*Endocrinology* 106: 375, 1980)

**E**STRADIOL (E) has been shown unequivocally to be a primary luteotropin in the rabbit for it alone maintains corpora lutea (CL) in the absence of any pituitary gonadotropin (1, 2). Although LH has been shown to affect rabbit CL, the mechanism of its luteotropic role remains obscure, since many of the luteotropic actions of LH can be accounted for by its stimulatory effect on follicular E production. One direct action of LH on rabbit CL is its stimulatory effect on adenylyl cyclase, an effect that can be elicited in a cell-free system in the absence of follicular elements (3–5). Subsequent to this stimulation, a refractory state (desensitization of the LH-stimulable adenylyl cyclase) appears in both follicles and

CL (6, 7). After the appearance of these refractory states, cessation of follicular steroidogenic activity occurs (leading to an interruption of E supply to the CL) (8) and functional luteolysis is initiated, as visualized by the cessation of luteal progesterone production (9). The question then arises whether the desensitization seen in CL and the ensuing luteolysis are due to a direct action of LH on the CL or whether the loss of LH-stimulable adenylyl cyclase and the cessation of steroidogenesis in CL are secondary to the interruption of E supply. To investigate some aspects of this problem and to study the effect of E on rabbit CL adenylyl cyclase, we treated pseudopregnant rabbits with a luteolytic (desensitizing) dose of hCG while at the same time providing exogenous E and determined the effects of these treatments both on the CL adenylyl cyclase system by measuring activity in homogenates and on CL function by measuring progesterone levels in CL and serum. In the present com-

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† To whom all correspondence and requests for reprints should be addressed.

munication, we report that E, even at a dose that maintains CL weight, does not prevent the luteolytic effect of hCG, and that treatment of control pseudopregnant rabbits with such doses of E results in unexpected and profound alterations in the LH-stimulable adenylyl cyclase of the rabbit CL. Preliminary accounts of these findings have been presented (10, 11).

## Materials and Methods

### Animals

New Zealand White rabbits (3.5–4.5 kg) which had littered at least once were housed in individual cages in air-conditioned quarters and allowed free access to water and a commercially pelleted food for at least 15 days before initiation of experiments. Such females, if not pregnant, were considered to be in estrus. Pseudopregnancy (PSP) was induced by injecting into the marginal ear vein 100 IU hCG (generously donated by Dr. J. Jewell, Ayerst Laboratories, Rouses Point, NY) dissolved in 0.5 ml 0.9% saline. The day after hCG injection was designated day 1 of PSP.

### Treatments and preparation of sera and CL homogenates

Starting at 2200 h on day 8 of PSP, rabbits received, at 12-h intervals, 0.1 ml sesame seed oil sc without (control) or with 1.5  $\mu$ g E (E-1.5 treatment) or 15  $\mu$ g E (E-15 treatment). The last injection was at 2200 h on day 11 of PSP, as shown in Fig. 1. Some rabbits received 100 IU hCG (0.5 ml 0.9% saline, iv) on day 9 of PSP between 1300–1500 h immediately after a blood sample had been taken.

The rabbits were bled from the marginal ear vein on days 9–12 of PSP between 1300–1500 h, except on day 12 when they were bled just before sacrifice. On day 12 of PSP, rabbits were sacrificed by cervical dislocation between 1000–1100 h. Ovaries were removed immediately and cooled to 0 C in iced Krebs-Ringer bicarbonate buffer prepared with half the recommended amount of  $\text{CaCl}_2$  (12). The original 12-day-old CL and, where appropriate, newly formed 3-day-old CL were dissected free of

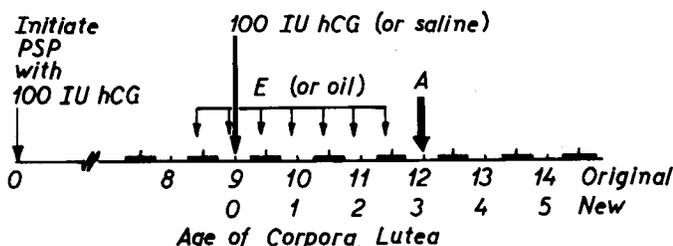


FIG. 1. Treatment schedule for the investigation of the effects of E on CL function and adenylyl cyclase activity in normal and hCG-injected rabbits. PSP was initiated in estrous New Zealand White rabbits (3.5–4.5 kg) that had littered at least once. Three groups of animals received 0.1 ml sesame oil (control), 1.5  $\mu$ g E in 0.1 ml sesame oil (E-1.5), or 15.0  $\mu$ g E in 0.1 ml sesame oil (E-15) twice daily sc. Injections were started at 2200 h on day 8 and terminated after 2200 h on day 11. Rabbits from each group received 0.5 ml saline with 100 IU hCG iv on day 9 between 1300–1500 h, and the remainder received 0.5 ml saline alone. On day 12, all rabbits were sacrificed, and their CL were dissected, weighed, homogenized, and assayed for adenylyl cyclase activities and progesterone content. A, Time of autopsy on day 12.

interstitial tissue using Graefe forceps (Roboz Surgical Co.) and kept in iced Krebs-Ringer bicarbonate buffer until further processing (30 min to 1 h). Before homogenization, CL were blotted and weighed. Homogenization was performed in 10 vol ice-cold 27% (wt/wt) sucrose in 1 mM EDTA and 10 mM Tris-HCl, pH 7.5, as previously described by Hunzicker-Dunn and Birnbaumer (5), followed by a 2-fold dilution with the same homogenizing medium. Homogenates were analyzed for adenylyl cyclase activity within 30 min and for progesterone content after storage at  $-20$  C for up to 6 months. Pooled 3- or 12-day-old CL from a single rabbit yielded enough homogenate for triplicate determinations each of basal, LH-stimulated, and isoproterenol-stimulated adenylyl cyclase activities as well as for duplicate determinations of protein and two triplicate assays of progesterone.

### Adenylyl cyclase assays

Adenylyl cyclase activity in 20- $\mu$ l aliquots of homogenate was determined, as described earlier (5), at 32.5 C in 50  $\mu$ l medium containing 3.0 mM [ $\alpha$ - $^{32}$ P]ATP ( $5$ – $15 \times 10^6$  cpm), 5.0 mM  $\text{MgCl}_2$ , 1 mM EDTA, 1 mM [ $^3$ H]cAMP ( $\sim 10,000$  cpm), 20 mM creatine phosphate, 0.2 mg/ml creatine kinase, 0.02 mg/ml myokinase, and 25 mM Tris-HCl. When present, LH was 10  $\mu$ g/ml and isoproterenol (Iso) was  $10^{-4}$  M. The final pH of the incubation (10 min) was 7.0. [ $^{32}$ P]cAMP formed was isolated by the method of Salomon *et al.* (13) as modified by Bockaert *et al.* (14). Under the conditions employed, adenylyl cyclase activities were linear with respect to time of incubation for up to 20 min and with respect to homogenate concentration for up to 40  $\mu$ eq of 1:20 homogenates (*i.e.* 20  $\mu$ l of homogenates prepared by homogenizing 1 part of CL in 10 parts of homogenization medium and omitting the subsequent 1:2 dilution step). Results are expressed as picomoles of cAMP formed per mg protein/min.

Protein was determined by the method of Lowry *et al.* (15) using bovine serum albumin (fraction V, Armour Pharmaceutical Co., Chicago, IL) as standard.

### Progesterone assays

Solutions, extractions of sera and homogenates, set-up of the RIA (based on antiserum GDN 337 kindly supplied by Dr. Gordon D. Niswender), separation of free from bound progesterone, estimation of recoveries, and evaluation of results were described in detail elsewhere (16). The characteristics of this antibody have been published by Gibori *et al.* (17) and confirmed by us.

Since serum progesterone was assayed in both CL homogenates and sera, we checked for interference by determining progesterone before and after an additional chromatography of the petroleum ether-extracted samples over Sephadex LH-20. Chromatography was performed according to Carr *et al.* (18) as described in Day and Birnbaumer (16). In two separate experiments, after correction for recoveries, we found no significant differences between values obtained on samples not subjected to chromatographic purification and on those subjected to the treatment. Values reported below were therefore obtained by subjecting aliquots of CL homogenates and sera to two petroleum ether extractions, evaporating to dryness under a stream of air, resuspending the residue in 500  $\mu$ l phosphate-buffered saline containing 0.1% gelatin (Difco Laboratories, Detroit, MI),

and assaying 10- to 100- $\mu$ l aliquots of this suspension for progesterone without further treatment. Coefficients of variation for interassay variability (3 serum pools; 11-14 assays) and intraassay variability (10-15 determinations/assay; 3 assays) were  $13.7 \pm 3.5$  and  $11.0 \pm 2.1$  (mean  $\pm$  SD), respectively (19). For further details, see Day and Birnbaumer (16).

### Materials

E (Sigma Chemical Co., St. Louis, MO) was dissolved in sesame seed oil (150  $\mu$ g/ml) for E-15 treatments and then diluted 1:10 with oil for E-1.5 treatments. LH (NIH-LH-S19, obtained through the Hormone Distribution Officer, NIAMDD) was kept as a stock solution (1 mg/ml) in 0.15 M NaCl. (-)-Iso was a gift from Dr. F. C. Nachod (Sterling Winthrop Research Institute, Newcastle upon Tyne, United Kingdom) and kept as a stock solution ( $10^{-1}$  M) in  $10^{-3}$  M HCl. For adenylyl cyclase assays, LH and Iso stock solutions were diluted with 0.1% bovine serum albumin to 50  $\mu$ g/ml and  $5 \times 10^{-4}$  M, respectively, 10  $\mu$ l of which were used in the assays. Creatine phosphate and creatine kinase were from Calbiochem (La Jolla, CA), and myokinase, ATP(Tris-salt), cAMP, EDTA, and Tris were from Sigma Chemical Co. [ $\alpha$ - $^{32}$ P]ATP (20-50 Ci/mmol) was synthesized and supplied by the Core Laboratory on Cyclic Nucleotide Research, Center for Population Research and Studies on Reproductive Biology, Baylor College of Medicine. [ $^3$ H]cAMP (10-15 Ci/mmol) was from Amersham/Searle Corp. (Arlington Heights, IL). All other chemicals and reagents were of the highest commercially available purity and were used without further purification.

Significance of differences between groups were calculated using Student's *t* test.

### Results

To explore whether both luteolysis and adenylyl cyclase desensitization in CL induced by hCG might be secondary to the interruption of follicular E supply, we subjected rabbits to the treatment schedule shown in Fig. 1 and described in *Materials and Methods*. Thus, we initiated E treatment on day 8 of PSP using two dose levels [1) 3  $\mu$ g E/day (two doses of 1.5  $\mu$ g at 12-h intervals) and 2) 30  $\mu$ g E/day (two doses of 15  $\mu$ g at 12-h intervals)] and continued this treatment throughout the time of study. A dose of 3  $\mu$ g E/day (E-1.5 treatment) is known to maintain CL in a functionally active state after hypophysectomy (2) or after irradiation of the ovary with x-rays, a treatment known to destroy the estrogen-producing follicles (1). A dose of 30  $\mu$ g E/day (E-15 treatment) was shown by Stormshak and Casida (20) to maintain CL weight after an otherwise luteolytic dose of LH. The effects of these treatments combined with an injection of 100 IU hCG on day 9, *i.e.* 15 h after initiation of E treatment, were studied.

#### *Effects of hCG in the absence and presence of exogenous E: supply on CL function and structure*

As seen in Fig. 2, neither E-1.5 nor E-15 affected serum levels of progesterone significantly when assayed

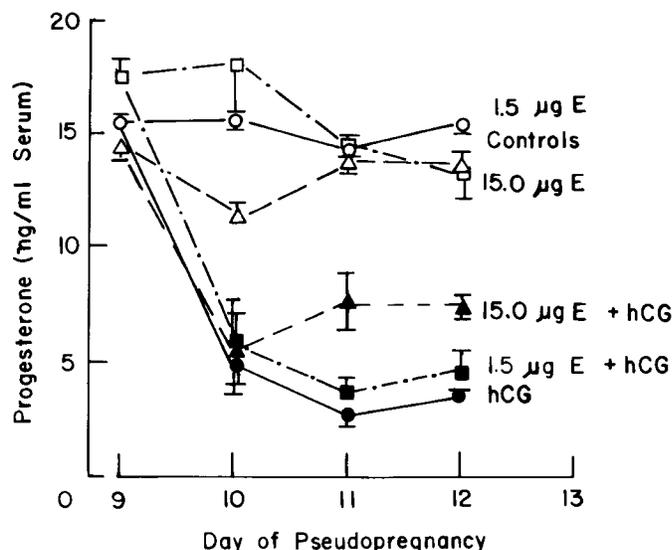


FIG. 2. Effect of E treatment alone or in combination with hCG treatment on serum progesterone levels in PSP rabbits. Animals treated as indicated in Fig. 1 were bled from the marginal ear vein between 1300-1500 h on days 9, 10, and 11 and between 0800-1000 h on day 12. Serum progesterone was measured by RIA, as described in the text. The mean  $\pm$  SEM are represented. The number of animals in each group is: days 9 and 12: control, 1.5  $\mu$ g E, and 15  $\mu$ g E,  $n = 9$  and hCG, 1.5  $\mu$ g E plus hCG, and 15  $\mu$ g E plus hCG,  $n = 6$ ; days 10 and 11: control, 1.5  $\mu$ g E, and 15  $\mu$ g E,  $n = 6$  and hCG, 1.5  $\mu$ g E plus hCG, and 15  $\mu$ g E plus hCG,  $n = 3$ . Relevant tests of significance: day 11: hCG vs. hCG plus E-15,  $P < 0.05$ ; day 12: hCG vs. hCG plus E-15,  $P < 0.01$ ; day 11: hCG vs. hCG plus E-1.5,  $P > 0.1$ ; and day 12: hCG vs. hCG plus E-1.5,  $P > 0.1$ . All hCG treatments differ from their respective controls with  $P < 0.005$ .

throughout the treatment period. The administration of hCG, however, resulted in rapid functional luteolysis, as seen by the drop in serum progesterone observed as early as 24 h after hCG injection. This lytic effect of hCG could not be prevented by either E-1.5 or E-15. Although a small rebound of serum progesterone occurred with E-30 treatment by day 12 of PSP, this increase with respect to hCG alone or hCG plus E-1.5 is small and further experiments are required to define its physiological implication.

While E-15 plus hCG treatments had little if any effect on serum progesterone levels, as seen on day 12 of PSP and compared to treatment with hCG alone, progesterone content in the original 12-day-old CL was clearly elevated in the E-15 plus hCG group when compared to either the group treated with hCG alone or the hCG plus E-1.5-treated group (Table 1). Although there was a tendency for progesterone levels in 12-day-old CL of the E-15 only group to be somewhat higher than those in control CL, the difference ( $P > 0.1$ ) was not significant (Table 1). Furthermore, tissue levels of progesterone in 12-day-old CL of E-15 plus hCG-treated rabbits did not differ significantly ( $P > 0.1$ ) from those in 12-day-old CL of control rabbits (Table 1).

Treatment with hCG alone resulted not only in func-

TABLE 1. Effect of E treatment on PSP rabbit CL weight and CL progesterone content in the presence or absence of hCG treatment

Treatment*	n	CL wt		CL progesterone	
		12 days old (mg/CL)	3 days old (mg/CL)	12 days old (ng/mg protein)	3 days old (ng/mg protein)
Oil	6	21.0 ± 0.5 <sup>b</sup>		135 ± 15 <sup>b</sup>	
Oil + hCG	3	8.6 ± 0.8 <sup>c</sup>	4.6 ± 0.2	12 ± 1 <sup>c</sup>	136 ± 11 <sup>d</sup>
E-1.5	6	19.1 ± 1.1 <sup>b</sup>		172 ± 36 <sup>e</sup>	
E-1.5 + hCG	3	8.2 ± 0.6 <sup>c</sup>	4.6 ± 0.4	26 ± 13 <sup>c</sup>	205 ± 10 <sup>f</sup>
E-15	6	21.9 ± 0.9 <sup>b</sup>		184 ± 17 <sup>f</sup>	
E-15 + hCG	3	16.1 ± 2.0 <sup>b</sup>	4.3 ± 0.2	123 ± 10 <sup>b</sup>	158 ± 15 <sup>b</sup>

\* When treated, rabbits received either hCG alone on day 9 or hCG on day 9 and E from 2200 h on day 8 through 2200 h on day 11. Animals were sacrificed on day 12. CL were dissected and weighed. For further details, see Fig. 1 and the text. Values represent the mean ± SEM of the number of animals (n) shown. Each determination was carried out with the CL of a different animal.

<sup>b</sup>  $P < 0.001$  vs. <sup>c</sup>;  $P > 0.1$  vs. <sup>d</sup>;  $P > 0.1$  vs. <sup>e</sup>;  $P > 0.05$  vs. <sup>f</sup>.

<sup>c</sup>  $P < 0.001$  vs. <sup>f</sup>.

<sup>d</sup>  $P < 0.025$  vs. <sup>f</sup>.

tional luteolysis but also in structural luteolysis, as evidenced by the loss of CL weight (Table 1). Contrary to the effects on progesterone production, E-15 but not E-1.5 protected the CL against hCG-induced weight loss (Table 1). Thus, in the presence of E-15, hCG was found to induce functional but not structural luteolysis. As evidenced by serum progesterone levels and CL weights, neither E-1.5 alone nor E-15 alone (*i.e.* no hCG treatment) appear to have any deleterious effect on rabbit CL of PSP.

Neither E-1.5 nor E-15 treatment had any effect on either weight or progesterone content of new 3-day-old CL formed in response to the hCG treatment. Mean weights and progesterone contents of these CL are shown in Table 1.

#### Effects of hCG in the absence and presence of exogenous E supply on the CL adenylyl cyclase system

The effects of E-1.5, and E-15 and hCG alone and in combination on CL adenylyl cyclase activities in 12-day-old as well as, where appropriate, in new 3-day-old CL are shown in Figs. 3 and 4.

Three activities are reported: basal, LH-stimulated, and Iso-stimulated. Although the luteal adenylyl cyclase system also responds to fluoride ion and prostaglandin E<sub>1</sub>, we chose to report on the Iso-stimulated activity because: 1) its magnitude is much closer to that of LH-stimulable activity than prostaglandin E<sub>1</sub>-stimulated activity, which at best is twice that of basal activity, and 2) Iso-stimulated activity depends on receptor-cyclase interactions which should be similar in principle to the interactions that lead to LH receptor-mediated activa-

tion of the adenylyl cyclase system; furthermore, the mode of action of fluoride as a stimulator is even less understood than that of hormones. Thus, Iso-stimulated activity represents a means of controlling whether changes that may occur at the level of LH-stimulated activity are, at the time of measurement, specific to the LH receptor-cyclase interaction or of a more general

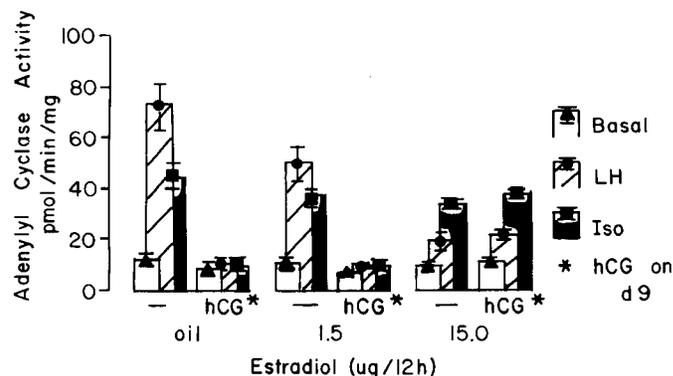


FIG. 3. Effect of E treatment on hormonally stimutable adenylyl cyclase in 12-day-old CL from PSP rabbits that received either saline or 100 IU hCG iv on day 9, as described in Fig. 1. After sacrifice on day 12, CL homogenates were prepared and assayed, as described in *Materials and Methods*. When present in the assay, LH (NIH-LH-S19) was 10  $\mu$ g/ml and Iso was  $10^{-4}$  M. The mean ± SEM are represented; there were three animals in each group. Tests of significance: stimulations by LH and Iso over basal in oil, E-1.5, E-15, and E-15 plus hCG,  $P < 0.001$ ; stimulations by LH and Iso in hCG and E-1.5 plus hCG,  $P < 0.05$ ; basal activities did not differ among themselves ( $P > 0.05$ ), except for hCG plus E-1.5 which was lower than the control (35%;  $P < 0.05$ ) and E-1.5 only groups (29%;  $P < 0.05$ ); LH-stimulated activities: oil vs. E-1.5,  $P > 0.05$ ; oil vs. E-15,  $P < 0.001$ ; oil vs. E-15 plus hCG,  $P > 0.0025$ ; E-15 vs. E-15 plus hCG,  $P > 0.35$ ; hCG vs. hCG plus E-15,  $P < 0.0025$ ; Iso-stimulated activities: oil vs. E-1.5 vs. E-15,  $P > 0.1$ ; E-15 vs. E-15 plus hCG,  $P > 0.1$ .

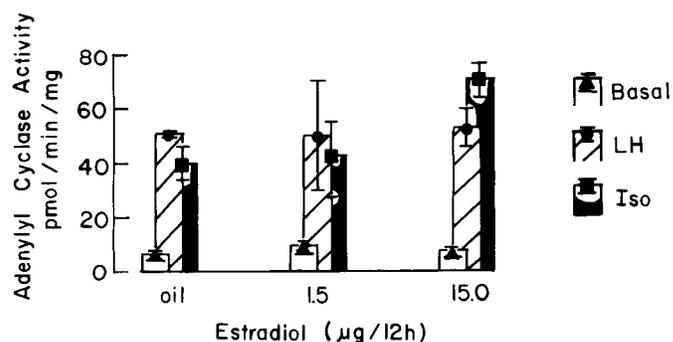


FIG. 4. Adenylyl cyclase activities in 3-day-old CL formed after injection of hCG (100 IU, iv) on day 9 into PSP rabbits treated or not treated with low (E-1.5) or high (E-15) doses of E. Newly formed CL were dissected from ovaries of 12-day PSP rabbits sacrificed between 1000-1100 h, homogenized, and assayed for basal, LH-stimulated, and Iso-stimulated adenylyl cyclase activities, as described in *Materials and Methods*. The mean ± SEM are represented; there were 3 animals in each group. Tests of significance: basal activities: oil vs. E-15,  $P > 0.1$ ; LH-stimulated activities: oil vs. E-1.5 vs. E-15,  $P > 0.1$ ; isoproterenol-stimulated activities: oil vs. E-1.5,  $P > 0.1$ ; oil vs. E-15,  $P > 0.05$ .

nature. As shown in Fig. 3, injection of hCG on day 9 resulted in the establishment 3 days later of a significant reduction or uncoupling of the cyclase system from any hormonal influence. Thus, hCG treatment resulted not only in a homologous desensitization (*i.e.* desensitization to itself) but also in a heterologous desensitization (21). Administration of E-1.5 *per se* did not significantly alter LH- or Iso-stimulable adenylyl cyclase activities, but neither did this treatment prevent the loss of hormone-stimulable adenylyl cyclase activities in response to hCG administration. The administration of E-15 alone resulted in a decrease in LH-stimulable activity ( $P < 0.001$ ) without a loss in Iso-stimulable activity ( $P > 0.05$ ). Interestingly, the decrease in LH-stimulable activity was partial (less than that obtained with hCG alone;  $P < 0.0025$ ) and appeared to be unaffected by additional treatment with hCG (LH in E-15 *vs.* LH in E-15 plus hCG,  $P > 0.35$ ). The additional treatment with hCG was also ineffective in reducing Iso-stimulable activity after E-15. Thus, E-15, while resulting in decreased LH responsiveness of adenylyl cyclase, seemed to protect against deleterious effects of hCG on the CL adenylyl cyclase system. As shown in Fig. 4, the CL induced by the luteolytic and desensitizing dose of hCG 3 days before the assay are very responsive to LH (7.8-fold stimulation) and to Iso (6.3-fold stimulation). Neither E-1.5 nor E-15 had a significant effect on the adenylyl cyclase system in the new 3-day-old CL.

#### Other

Unless hCG had been given, no new ovulations were observed.

#### Discussion

It has been argued (for a recent discussion, see Ref. 22) that since most of the systemic E originates in the ovary, the reason low doses of E may be without apparent effect on ovarian function, including CL function, may be because the actual physiological levels in ovaries (and, hence, CL) are much higher than the physiological levels in tissues distal to the ovary, such as the uterus and hypothalamus. While this argument seems to be supported by the work of Younglai (23) and Patwardham and Louthier (24), who found very high levels of E in rabbit follicular fluid and elevation of follicular tissue E shortly after LH administration, two types of counterarguments need to be considered. 1) The affinities determined by Richards for E binding to rat CL estrogen receptor are similar (in the  $10^{-9}$ M range) to those determined by Clark *et al.* for rat uterine estrogen receptor (25, 26). 2) As alluded to previously in this report, both Keyes and Nalbandov (1) and Rennie (2) have shown that doses of 0.1–0.2  $\mu$ g twice daily are sufficient in the

rabbit to maintain normal CL in x-irradiated ovaries and transplanted CL in hypophysectomized and ovariectomized animals. These findings strongly argue that if a luteolytic action of hCG were due only to the interruption of the follicular E supply to the CL, replacement therapy with 0.15  $\mu$ g/twice daily should have been adequate to maintain these CL. Since treatment with this low dose of E, initiated 15 h before the administration of hCG, did not prevent the luteolytic effect of hCG, we conclude that this action of hCG is not due to a mere interruption of the follicular E supply. Thus, at least three effects of hCG (and, by inference, of LH) appear to be due to the direct interaction of the gonadotropin with the CL: stimulation of adenylyl cyclase, desensitization of adenylyl cyclase, and functional luteolysis (interruption of progesterone production by the CL). On a mechanistic basis, it is not known whether the shutdown of progesterone production is an effect independent of effects on adenylyl cyclase or is a consequence of effects on adenylyl cyclase.

The combined treatment of E-15 plus hCG allowed for a dissociation in the rabbit between functional luteolysis and structural luteolysis, since E-15-treated CL 3 days after hCG had maintained their weights but lost their normal capacities to secrete progesterone. The data do not address themselves to the interesting question of whether the functional decrease in progesterone-secreting activity might be reversible under some circumstances. Of potential interest in this respect is the partial return of serum progesterone in hCG plus E-15-treated rabbits as opposed to hCG alone- or hCG plus E-1.5-treated animals. Although, as mentioned earlier, further research is needed to substantiate this difference (significant at the  $P < 0.01$  level in the experiment shown in this report), it should be noted that this difference is not likely to be due to early activation of new CL by E, since at this time they are not yet dependent on E (27) and do not yet contain more than about 10% of their normal complement of cytosolic E receptors (28). Experiments are currently underway to determine whether prolonging the E treatment or altering the dose schedule will result in the reversal of the effect of hCG on serum progesterone levels. In the rat, functionally inactive but structurally intact CL have been observed, as seen in ergocryptine (CB-154)-treated cycling rats (29). Similarly, Spies *et al.* (30) showed that PSP rabbits with uteri traumatized on days 4 or 8 retained for 21 days CL structures that did not secrete progesterone unless estrogen was administered throughout. More recently, Bender and coworkers (31) showed that CL of PSP rabbits previously subjected to exogenous E treatment (silastic capsule implant) stopped making progesterone upon E withdrawal but could be reactivated with E up to 2 days later, indicating that here also a dissociation between functional luteolysis and structural luteolysis had occurred. These findings

coupled with data presented in this report showing the presence of structurally intact but functionally much less active CL in E-15 plus hCG-treated rabbits clearly indicate that these two aspects of luteolysis are events that should be considered separately. It is of interest to note that the CL of the pregnant monkey stops producing progesterone during the first week of pregnancy (functional luteolysis) but resumes activity at the time of delivery, indicating that cell and organ integrities had been maintained in spite of a prolonged shutdown of steroidogenesis (32, 33).

hCG plus E-15-treated CL did not secrete much more progesterone than hCG only- or hCG plus E-1.5-treated CL, but their progesterone content was normal, *i.e.* not different from that of control ( $P > 0.1$ ) or E-15 alone-treated CL ( $P > 0.05$ ). A fall in serum progesterone can arise from a decrease in progesterone synthesis, an inhibition of secretion, or a combination of both. The data appear to indicate that the last possibility applies after E-15 plus hCG treatment, since serum progesterone levels were low even though intracellular levels were the same as those in actively secreting control CL ( $P > 0.1$ ). This suggests that LH and hCG, through stimulation of adenylyl cyclase and regulation of cAMP levels, may not only modulate luteal progesterone synthesis, as shown in the bovine CL by Marsh and collaborators (34), but may also and independently modulate progesterone secretion. It should be noted that cAMP is known for stimulating the release of secretory vesicles via exocytosis in both endocrine and exocrine glands, as seen in the stimulation of insulin secretion from pancreatic islets (35) and of amylase secretion from parotid glands (36). Furthermore, Rubin's laboratory (37, 38) studying the stimulatory effect of ACTH on cat adrenal fasciculata cells has shown the appearance, upon hormonal stimulation, of high density granules that may be of the secretory type and of protein secretion concomitant with steroid release. The assumption that steroid hormones such as adrenal corticoids and CL progesterone exit cells by simple diffusion (39) rather than via exocytosis of secretory vesicles has by no means been adequately documented. It may be that the hCG plus E-15-treated CL is an organ in which the regulation of the secretory process by LH-stimulable adenylyl cyclase has become limited due to desensitization and in which the steroidogenic machinery, assumed to be dependent on E, is fully operative, although feedback inhibited by accumulated progesterone.

Although E-15 treatment did not result in an alteration in progesterone secretion by CL, it did have a profound action on the LH-stimulable adenylyl cyclase and its response to hCG treatment. Our finding that no effect on serum progesterone is seen after treatment with E alone confirms data reported by Holt *et al.* (40). The means by which E-15 treatment resulted in a selective decrease in

LH-stimulable adenylyl cyclase is not clear. One possibility is that estrogen treatment initiates an endogenous LH surge, and that the effect of a single short lived LH peak differs from the effect of the more prolonged exposure that occurs when hCG is given. Sawyer *et al.* (41) demonstrated that within 52 h after estrogen injection in progesterone-treated animals, 40% of the treated animals ovulated. Others have shown that a single estrogen injection results in a subovulatory LH peak (42, 43). Since previous studies from this laboratory suggested that ovulatory doses of hCG and LH were necessary for desensitization (7) and since we observed no new ovulatory points in E-15-treated animals, this possibility seems unlikely but bears further investigation. Another possibility is that estrogen exerts a direct effect on the CL through a receptor-mediated event. Cytoplasmic estrogen receptors have been demonstrated in rabbit CL as early as days 3-4 of PSP (28). This appearance of receptors slightly precedes the development of estrogen dependence in the CL (27). The fact that the original CL are affected by the elevated estrogen treatment while the CL newly induced by hCG are not is consistent with what is known about the appearance of cytoplasmic receptors. That the 3-day-old CL were not altered by estrogen treatment is evidenced by the similarity between hormonally stimulable adenylyl cyclase activities, CL weights, and luteal progesterone levels seen in the control and E-treated groups.

The present experiments do not provide evidence as to whether E-1.5 or E-15 have any early effects on the CL and might not have interfered with the normal course of action of hCG on hormonal responsiveness of CL adenylyl cyclase. One possibility is that E-1.5 may have protected against hCG-induced homologous desensitization (*i.e.* loss of LH stimulability without loss of Iso stimulability). In this instance, the loss of responsiveness to both hormones, as observed 3 days later (Fig. 3), would be a secondary consequence to the luteolytic process initiated by hCG but not dependent on an initial homologous desensitization reaction. Another possibility is that the E-15 treatment, rather than truly protecting against the initial homologous desensitization, only protected against the general loss of hormone stimulability (secondary to protection against structural luteolysis). The LH stimulability of adenylyl cyclase seen 3 days after hCG in CL of E-15-treated animals would then represent an early rebound rather than a protection. Clearly, further investigations are warranted into the effects of high and low doses of estrogen on rabbit CL function.

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