

Prostaglandins Do Not Appear to Play a Role in hCG-Induced Regression or Desensitization of Rabbit Corpora Lutea¹

HOWARD J. KIRCHICK² and LUTZ BIRNBAUMER

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

ABSTRACT

Prostaglandin $F_{2\alpha}$ has been suggested to be a physiological luteolysin in several species. It was the intent of the study reported herein to determine whether prostaglandins are involved in the hCG-induced desensitization of the luteal LH-responsive adenylyl cyclase or hCG-induced luteal regression. Rabbits were made pseudopregnant by injection of 75 IU of hCG (Day 0 of pseudopregnancy). Rabbits ($n = 34$) were surgically implanted with estradiol-filled Silastic capsules or empty capsules on Day 5 of pseudopregnancy. Other rabbits ($n = 16$) did not receive implants of any kind. On Day 6 of pseudopregnancy, all rabbits were injected with oil or indomethacin (70 mg) at 1000 h and with saline or hCG (75 IU) at 1600 h; at 1800 h, the oil and indomethacin injections were repeated. At 1000 h on Day 7 of pseudopregnancy (18 h after injection with hCG or saline), the rabbits without implants were killed, blood was collected by cardiac puncture, and the ovaries were removed and corpora lutea dissected free. Luteal homogenates were assayed for adenylyl cyclase activity and progesterone content, and the serum obtained was assayed for progesterone. Blood was drawn at 1600 h (24 h after hCG or saline treatment) from the rabbits with estradiol-filled or empty implants. The serum obtained was assayed for progesterone. Injection of hCG caused an increase in basal luteal adenylyl cyclase activity but caused decreases in LH-, isoproterenol-, and NaF-stimulated luteal adenylyl cyclase activities as well as a decrease in luteal progesterone concentration. Indomethacin treatment alone had no major effect on any parameter measured, but did cause an attenuation of the increased basal adenylyl cyclase activity found after hCG injection. Treatment with indomethacin did not prevent hCG-induced desensitization or luteal regression. Estradiol, on the other hand, partially protected the corpora lutea from the hCG-induced decrease in serum progesterone.

INTRODUCTION

Although much is known about what maintains the rabbit corpus luteum (CL), the causes of luteolysis in the rabbit are not known. Several avenues of research, however, have provided clues to the control of luteolysis. It has been reported that hysterectomy will prolong the life of the CL in pseudopregnant rabbits which implicates a uterine factor as a luteolysin (Scott and Rennie, 1970). Spies et al. (1968a,b) have reported that uterine trauma will also extend the life of the CL. Hunzicker-Dunn and Birnbaumer (1976a) have found that the LH-stimulable adenylyl cyclase activity of CL is higher in hysterectomized pseudopreg-

nant rabbits than in intact pseudopregnant rabbits. There have been many reports suggesting that prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) is the uterine luteolysin in several species including the rabbit (Scott and Rennie, 1970; McCrackan et al., 1973; O'Grady et al., 1972; Marsh, 1971; Hichens et al., 1974; Chang and Hunt, 1972; Dunn et al., 1973; Labhsetwar and Watson, 1974; Shaikh et al., 1977; Greenwich et al., 1976; Torjesen et al., 1978; Behrman et al., 1976; Keyes and Bullock, 1974; Goding et al., 1971, 72; Carlson and Gole, 1978). However, Scott and Rennie (1970) have also reported that CL from Day 2 pseudopregnant rabbits transplanted under the kidney capsule of Day 12 pseudopregnant rabbits will regress at approximately the same time as the in situ CL at the end of pseudopregnancy. This evidence makes the prospect of uterine $PGF_{2\alpha}$ as the luteolysin in rabbits doubtful since the $PGF_{2\alpha}$ would have to reach the ectopic CL via the systemic circulation. Hence, very large amounts of $PGF_{2\alpha}$ would have to be secreted by the

Accepted February 3, 1981.

Received November 7, 1980.

¹ This work was supported by NIH grant HD-09581.

² Reprint requests. Recipient of individual NIH postdoctoral fellowship HD-05823.

uterus to have an effect on the ectopic CL. Increases in peripheral serum PGF_{2α} levels toward the end of pregnancy or pseudopregnancy have not been reported. To the contrary, it has been reported (Carlson and Gole, 1978) that there is no change in peripheral serum PGF_{2α} during pseudopregnancy in rabbits. Therefore, the uterine luteolysis is probably something other than PGF_{2α} which is not cleared as rapidly as prostaglandins are.

This does not preclude the possibility that PGF_{2α} is a physiological luteolysin in the rabbit since indomethacin, a potent inhibitor of PGF_{2α} synthesis, has been reported to prolong pseudopregnancy (O'Grady et al., 1972; Carlson and Gole, 1978). Since the source of the PGF_{2α} is not likely to be the uterus, its source may be the corpus luteum itself. The role of the uterine factor may be to stimulate luteal PGF_{2α} synthesis. Demers et al. (1973) have demonstrated that the CL of the rat are capable of synthesizing PGF_{2α} in vitro in response to LH and PGE₂. The results from Demers' study suggest the possibility that hCG/LH-induced luteal regression could be a result of increased PGF_{2α} synthesis by CL in response to hCG or LH.

Therefore, the purpose of this study was to determine whether an inhibitor of prostaglandin synthesis (indomethacin) could prevent hCG-induced luteal regression and adenylyl cyclase desensitization. Since the CL are dependent upon estradiol for survival and since hCG-induced ovulation and/or luteinization temporarily interrupts the supply of estradiol, any beneficial effects of indomethacin following hCG injection could be masked by the consequent fall in estradiol secretion. Hence, some of the experiments reported herein were performed using estradiol-filled Silastic implants to offset any loss of estradiol secretion.

MATERIALS AND METHODS

Materials

Indomethacin (Sigma Chemical Co.) was suspended in sesame oil containing ethanol (30%) at a concentration of 35 mg/ml. Human chorionic gonadotropin (hCG) was a gift from Dr. John B. Jewell (Ayerst Labs.) and was dissolved in 0.15 M saline to yield a concentration of 250 IU/ml. LH (NIH-LH-S19, obtained from NIAMDD) was kept as a stock solution (1 mg/ml) in 0.15 M NaCl. (-)-Isoproterenol was a gift from Dr. F. C. Nachod (Sterling Winthrop Research Institute) and kept as a 10⁻² M stock solution in 10⁻³ M HCl. NaF (Fisher Scientific Co.) was kept as a 1 M stock solution. Prostaglandin E₁ (PGE₁), kept as a 1

mg/ml stock solution in 10 mM Tris-base, was a gift from Dr. John E. Pike (Upjohn Co.) as was the progesterone-11-hemisuccinate-tyrosine-methyl-ester. For adenylyl cyclase assays, LH, isoproterenol, NaF, and PGE₁ stock solutions were diluted with water to 50 μg/ml, 500 μM, 50 mM, and 50 μg/ml, respectively; 10 μl of each solution was used in the assays. Creatine phosphate and creatine kinase were from Calbiochem; myokinase, ATP (Tris-salt), cAMP, EDTA, and Tris were from Sigma Chemical Co. [α-³²P] ATP (20–50 Ci/mmol), synthesized according to Walseth and Johnson (1979) and purified according to Birnbaumer et al. (1979), was supplied by the Core Laboratory on Cyclic Nucleotide Research, Center for Population Research and Studies on Reproductive Biology, Baylor College of Medicine, TX. [³H] cAMP (10–15 Ci/mmol) was from Amersham Searle. All other chemicals and reagents were of the highest commercially available purity and were used without further purification.

Animals

Virgin New Zealand White rabbits (3–4 kg) were housed in individual cages in air-conditioned quarters and were fed Purina rabbit chow ad libitum for at least 15 days before initiation of experiments. Pseudopregnancy was induced by i.v. injection of 75 IU hCG (0.3 ml). The day following hCG injection was designated Day 1 of pseudopregnancy.

Treatments and Preparation of Sera and CL Homogenates

Experiment 1. On Day 6 of pseudopregnancy, the rabbits were divided into four groups (4 animals/group). At 1000 h, the rabbits in groups 3 and 4 were given a 2 ml s.c. injection of the indomethacin suspension (70 mg/rabbit) while the animals in groups 1 and 2 received the oil-ethanol injection vehicle without indomethacin. At 1600 h, the rabbits in groups 2 and 4 were given a 0.3 ml i.v. injection of hCG (75 IU) while those in groups 1 and 3 received saline only. At 1800 h, the indomethacin and vehicle injections were repeated. At 1000 h on Day 7 of pseudopregnancy (18 h after hCG or saline treatment) the rabbits were killed by cervical dislocation, blood was collected by cardiac puncture, and the ovaries were removed and placed in ice-cold Krebs-Ringer bicarbonate buffer prepared with one-half the recommended amount of CaCl₂ (Cohen, 1957). The blood was allowed to clot at room temperature for about 10 min and was placed in a refrigerator for 4 h after which it was spun in a refrigerated centrifuge for 10 min to obtain serum. The corpora lutea were dissected free of the ovaries, cleaned of adhering interstitial tissue and kept in ice-cold Krebs-Ringer bicarbonate buffer until further processing (30 min–1 h). Prior to homogenization, CL were blotted and weighed. Homogenization was performed in 10 volumes of ice-cold 27% w/w sucrose in 10 mM Tris-HCl, 1 mM EDTA, pH 7.5, as described by Hunzicker-Dunn and Birnbaumer (1976a), followed by a twofold 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 2 weeks. Homogenates were also assayed for protein (Lowry et al.,

1951) using bovine serum albumin (Fraction V, Armour) as standard.

Experiment 2. A second experiment was performed which differed from experiment 1 as follows. Eighteen rabbits had estradiol-filled Silastic capsules (3.35 mm i.d., 4.65 mm o.d.; 12 mm length) surgically implanted according to Holt et al. (1975) at 1000 h of Day 5 of pseudopregnancy. The remaining 16 rabbits received empty implants. Estradiol-filled Silastic implants of this type resulted in serum estradiol concentrations of ~ 14 pg/ml in normal pseudopregnant rabbits (unpublished data). Four groups were established as in experiment 1 for both estradiol-treated and control rabbits. All injections followed the same schedule as in experiment 1. At 1600 h on Day 7 of pseudopregnancy (24 h after hCG or saline treatment), 2–3 ml of blood were drawn from the marginal ear vein of each animal. Serum samples were derived as described above, frozen, and stored at -20°C for approximately 1 week, and were then assayed for progesterone.

Adenylyl Cyclase Assays

Adenylyl cyclase activity in 20 μl aliquots of homogenates was determined as described (Hunzicker-Dunn and Birnbaumer, 1976a) at 32.5°C in 50 μl medium containing 3.0 mM [α - ^{32}P] ATP ($\sim 5 \times 10^6$ cpm), 5.0 mM MgCl_2 , 1 mM EDTA, 1 mM [^3H]-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 $\mu\text{g}/\text{ml}$, isoproterenol was 100 μM , NaF was 10 mM, and PGE₁ was 10 $\mu\text{g}/\text{ml}$. The final pH of the incubation (10 min) was 7.0. The [^{32}P]cAMP formed was isolated by the method of Salomon et al. (1974) as modified by Bockaert et al. (1976). 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 the equivalent of 40 μl of 1:20 homogenates (i.e., 20 μl 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 pmoles of cAMP formed/min/mg protein.

Progesterone Assays

To determine procedural losses, [$1,2$ - ^3H] progesterone (New England Nuclear, $\sim 1.5 \times 10^3$ cpm, sp act 50 Ci/mmol) was added to all serum and homogenate samples. The samples were then extracted with 10 volumes of petroleum ether. The dried extracts were reconstituted with 1.2 ml of 0.1% gelatin–0.01 M phosphate-buffered saline (gel-PBS), 0.2 ml of which were counted in a liquid scintillation counter to determine recovery.

The assay was performed as follows. The samples and standards (Calbiochem) were pipetted in duplicate to 12 \times 75 mm assay tubes and were brought to 500 μl with gel-PBS. Antiserum GDN #337 (supplied by Dr. G. D. Niswender) (200 μl) at a dilution of 1:80,000 in 1:100 NRS (1 part normal rabbit serum plus 99 parts gel-PBS) and 100 μl of ^{125}I -progesterone-11-hemisuccinate-tyrosine-methyl-ester ($\sim 20,000$ cpm) were added simultaneously to each tube containing either sample or standard at room temperature. In addition, three tubes received 100 μl of labeled progesterone

plus 1.7 ml of gel-PBS (total count tubes) and three tubes with 500 μl of gel-PBS received 200 μl of 1:100 NRS and 100 μl of labeled hormone (nonspecific binding tubes). All tubes were transferred to a cold room where they were incubated overnight. The following day 1 ml of 0.5% charcoal–0.05% dextran was added in the cold room to each tube (except the three "total-count" tubes). Following a 15 min incubation in the cold, the tubes were spun for 15 min in a refrigerated centrifuge, and the supernatants were poured into clean tubes and counted for 1 min each in a Searle 1197 gamma counter. Assay results were analyzed using a computer program based on the assay statistics described by Midgely et al. (1969) and Duddleson et al. (1972). Cross reactivities of various other steroids with the GDN #337 antiserum using this assay procedure are not significantly different from those described by Gibori et al. (1977) using tritiated progesterone as labeled hormone. The amount of [^3H]progesterone added to determine recovery was subtracted from the values obtained in the assay. The "zero" competition tubes for this procedure give $25 \pm 1\%$ binding, and the 50% inhibition point is 410 ± 26 pg/tube. The assay sensitivity is 7 ± 3 pg/tube, and the slope of the standard curve is -1.76 ± 0.04 . These values are the means \pm SEM of nine assays.

Statistics

Multiple comparisons were made using analysis of variance (ANOVA). Where noted, Student's *t* test was used. Differences were considered significant when $P < 0.05$.

RESULTS

Effects of Indomethacin on hCG-Induced Ovulation

The timing of injections and dose of indomethacin used were sufficient to block the ovulations which normally occur in pseudo-pregnant rabbits injected with 75 IU of hCG. This is based on the observation that whereas there were numerous ovulation points on the ovaries of oil-treated hCG-injected rabbits, there were none on the ovaries of indomethacin-treated hCG-injected rabbits. The dose used has also been reported by others to be effective in blocking hCG-induced ovulation (Hunzicker-Dunn and Birnbaumer, 1976; O'Grady et al., 1972).

Effects of Indomethacin on hCG-Induced Desensitization of Luteal Adenylyl Cyclase

The results of the adenylyl cyclase assays are shown in Table 1. Injection of hCG significantly elevates ($P < 0.005$) basal luteal cyclase activity by 42% in indomethacin-treated rabbits and by 71% in oil-treated rabbits when compared with saline-injected controls. Indomethacin alone does not decrease basal cyclase

TABLE 1. Effects of indomethacin on hCG-induced desensitization of luteal adenylyl cyclase activities (mean ± SEM in pmoles/min/mg).

Treatment	n	Additions to assay*				
		None	LH	ISO	NaF	PGE ₁
Oil:saline	4	9.8 ± 1.1 ^a	49.4 ± 5.0 ^a	29.7 ± 1.4 ^a	77.8 ± 1.8 ^a	28.1 ± 2.1
Indomethacin:saline	4	8.2 ± 0.5 ^a	52.9 ± 2.6 ^a	29.6 ± 2.2 ^a	71.4 ± 4.5 ^a	32.7 ± 1.7
Oil:hCG	4	16.7 ± 0.5 ^b	20.7 ± 0.6 ^b	25.8 ± 1.1 ^b	62.5 ± 1.8 ^b	33.1 ± 1.7
Indomethacin:hCG	4	11.6 ± 0.9 ^c	14.3 ± 0.7 ^c	21.4 ± 0.6 ^c	51.6 ± 3.2 ^c	30.2 ± 2.5

*Groups with different superscripts are different at at least the P<0.05 level.

activity, nor does it alter LH-, isoproterenol-, NaF-, or PGE₁-stimulated adenylyl cyclase activities. Injection of hCG decreases adenylyl cyclase activities in the presence of LH, isoproterenol, and NaF by 58% (P<0.005), 13% (P<0.05), and 20% (P<0.005), respectively, in animals not treated with indomethacin, and by 73% (P<0.0005), 27% (P<0.005), and 28% (P<0.0005), respectively, in indomethacin-treated animals. Injection of hCG has no effect on PGE₁-responsive cyclase activity. The levels to which the LH-, isoproterenol-, and NaF-stimulated cyclase activities fall after hCG injection are all significantly lower in indomethacin-treated rabbits than in oil-treated rabbits (P<0.0005, 0.05, and 0.025, respectively). The statistical evaluation of the comparisons were all made using ANOVA except for those in the LH-responsive cyclase group. For this group Student's t test was used because of nonhomogeneity of variances.

Effects of Indomethacin on the hCG-Induced Decrease in Serum and Luteal Progesterone

The serum and tissue progesterone data are shown in Fig. 1. Indomethacin alone has no effect on either serum or tissue progesterone. Injection of hCG causes a decrease in tissue progesterone regardless of the presence (40% decrease; P<0.005) or absence (42% decrease; P<0.005) of indomethacin. However, serum progesterone is significantly decreased (P<0.025) in only the indomethacin-treated rabbits following hCG injection (46% decrease).

Effect of Exogenous Estradiol on the hCG-Induced Decrease in Serum Progesterone

By 24 h after hCG injection, as opposed to 18 h after injection, serum progesterone is significantly decreased regardless of whether

indomethacin was injected (Table 2). Although exogenous estradiol does not prevent the hCG-induced decline in serum progesterone, the decrease is less in the presence of exogenous estradiol than in its absence.

DISCUSSION

Our data lead us to the conclusion that prostaglandins do not play a major role in hCG-induced luteal regression or hCG-induced desensitization of the LH-responsive luteal adenylyl cyclase. The most compelling evidence we have for this conclusion is that a dose of indomethacin capable of blocking ovulation, a PG-dependent event (Armstrong and Ginwich, 1972; Grinwich et al., 1972; Armstrong et al., 1974), did not prevent a fall in both serum (despite the presence of estradiol implants) and tissue progesterone following hCG injection, nor did it prevent desensitization of the LH-responsive adenylyl cyclase. Several interesting effects of indomethacin were uncovered however. Although indomethacin had no effect on acute stimulation (hormone-stimulated cyclase activity in saline-injected rabbits) of the luteal adenylyl cyclase by LH (Table 1), it did have an apparent effect on chronic stimulation (basal cyclase activities in hCG-injected rabbits). We expected basal adenylyl cyclase activity to be elevated following hCG administration since this activity in actuality was hormone-stimulated. However, we did not expect indomethacin treatment to attenuate this elevation. Furthermore, the data imply that indomethacin also decreased LH-, isoproterenol-, and NaF-stimulated adenylyl cyclase activities after hCG injection, but had no effect on PGE₁-stimulated adenylyl cyclase activity.

The data suggest, therefore, the possibility that prostaglandins may have an effect on the

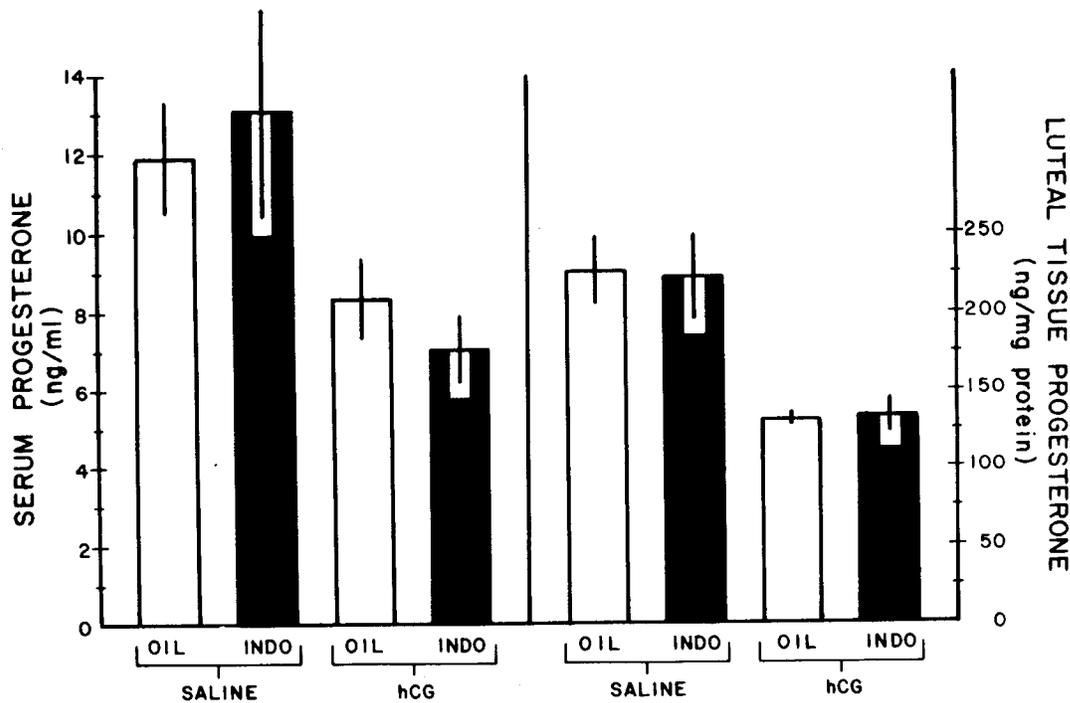


FIG. 1. Effects of indomethacin on serum and luteal tissue progesterone of hCG-treated pseudopregnant rabbits. Rabbits were treated with either oil or indomethacin 6 h before and 2 h after injection of either saline or hCG on Day 6 of pseudopregnancy. Rabbits were killed 18 h after receiving saline or hCG, and serum progesterone (ng/ml; left panel) and luteal tissue progesterone (ng/mg protein; right panel) were measured. Oil-treated groups are represented by open bars and indomethacin-treated groups by solid bars. Each bar represents the mean \pm SEM of four rabbits.

timing of the desensitization process. Further evidence in support of this hypothesis comes from the serum progesterone data. We did not expect drastic falls in serum progesterone since the animals were killed only 18 h after hCG injection, a time at which the luteolytic response to hCG is still in its early stages. However, it is interesting that only the decrease in

the indomethacin-treated group was significant.

That both the LH- and the isoproterenol-responsive luteal adenylyl cyclase activities are desensitized by hCG/LH injection, albeit to different degrees, has been reported by others (Harwood et al., 1979, 1980; Day et al., 1979; Hunzicker-Dunn et al., 1979; Day and Birnbaumer, 1980; Fletcher et al., 1980) as well as

TABLE 2. Effects of estradiol and indomethacin on the hCG-induced fall in serum progesterone [mean \pm SEM (n) of serum progesterone in ng/ml 24 h after hCG].

	No E ₂ treatment*		E ₂ treatment*	
	Saline	hCG	Saline	hCG
Oil	11.4 \pm 0.6 ^a (4)	4.8 \pm 0.8 ^{bc} (4)	9.6 \pm 0.6 ^a (4)	6.4 \pm 1.0 ^b (5)
Indomethacin	11.7 \pm 0.9 ^a (4)	4.1 \pm 0.7 ^c (4)	11.5 \pm 1.3 ^a (4)	6.4 \pm 0.7 ^b (5)
Total	11.5 \pm 0.5 ^d (8)	4.4 \pm 0.5 ^f (8)	10.6 \pm 0.8 ^d (8)	6.4 \pm 0.6 ^e (10)

*Groups with different superscripts (a-c) are significantly different at at least $P < 0.05$, and groups with different superscripts (d-f) are significantly different at at least $P < 0.05$.

that the PGE₁-responsive adenylyl cyclase is not affected by hCG/LH injection (Hunzicker-Dunn and Birnbaumer, 1976b; Fletcher et al., 1980). These data suggest that LH and isoproterenol may act via similar adenylyl cyclases or coupling mechanisms whereas PGE₁ either acts on a different adenylyl cyclase (either in the same cells or in a different cell type) or else that its receptors couple to the adenylyl cyclase in a manner different from those for LH and isoproterenol.

In a previous study from this laboratory (Day and Birnbaumer, 1980), it was reported that estradiol treatment suppressed the LH-responsive luteal adenylyl cyclase, and in addition caused the serum progesterone concentrations following hCG injection to remain somewhat elevated. The results from the present study confirm these previous findings concerning serum progesterone. It is our belief that the exogenous estradiol either causes a decrease in LH receptor number or affinity or causes a partial uncoupling of the LH receptor from the adenylyl cyclase system. This hypothesis would explain both the decrease in LH-responsive adenylyl cyclase and the partial protection of the corpora lutea from hCG-induced desensitization and luteal regression.

In summary, the use of a prostaglandin synthetase inhibitor in quantities sufficient to block ovulation does not prevent hCG-induced desensitization and luteal regression regardless of the presence or absence of exogenous estradiol. Hunzicker-Dunn and Birnbaumer (1976a) reported similar findings using estrous rabbit follicles: i.e., indomethacin did not block hCG-induced desensitization. In addition, the desensitization found appears to be heterologous, but not all-inclusive. Finally, exogenous estradiol, but not indomethacin, appears to protect partially the corpora lutea from functional regression.

ACKNOWLEDGMENTS

We wish to thank Cathleen Doherty for her able work as technician and Mick Scheib for efficient secretarial aid in the preparation of the manuscript. We also wish to thank Dr. Joel Abramowitz for his valuable input into these studies and Dr. Theodore Swartz who has the uncanny knack of keeping the laboratory supplied with [α -³²P] ATP.

REFERENCES

- Armstrong, D. T. and Grinwich, D. L. (1972). Blockade of spontaneous and LH-induced ovulation in rats by indomethacin, an inhibitor of prostaglandin biosynthesis. *Prostaglandins* 1, 21-28.
- Armstrong, D. T., Grinwich, D. L., Moon, Y. S. and Zamecnik, J. (1974). Inhibition of ovulation in rabbits by intrafollicular injection of indomethacin and prostaglandin F antiserum. *Life Sci.* 14, 129-140.
- Behrman, H. R., Grinwich, D. L. and Hichens, M. (1976). Studies on the mechanism of PGF_{2α} and gonadotropin interactions on LH receptor function in corpora lutea during luteolysis. *Adv. Prostaglandin Thromboxane Res.* 2, 655-666.
- Birnbaumer, L., Torres, H. N., Flawia, M. M. and Fricke, R. F. (1979). Improved methods for determination of guanylyl cyclase activity and synthesis of [α -³²P]GTP. *Anal. Biochem.* 92, 405-411.
- Bockaert, J., Hunzicker-Dunn, M. and Birnbaumer, L. (1976). Hormone-stimulated desensitization of hormone-dependent adenylyl cyclase: Dual action of luteinizing hormone on pig Graafian follicle membranes. *J. Biol. Chem.* 251, 2653-2663.
- Carlson, J. C. and Gole, J.W.D. (1978). CL regression in the pseudopregnant rabbit and the effects of treatment with prostaglandin F_{2α} and arachidonic acid. *J. Reprod. Fertil.* 53, 381-387.
- Chang, M. C. and Hunt, D. M. (1972). Effect of prostaglandin F_{2α} on the early pregnancy of rabbits. *Nature* 236, 120.
- Cohen, P. P. (1957). Suspending media for animal tissues. In: *Manometric Techniques*. (W. W. Umbreit, R. H. Burris and J. F. Stauffer, eds.). Burgess Publishing Co., Minneapolis. pp. 147-150.
- Day, S. L., Abramowitz, J., Hunzicker-Dunn, M. and Birnbaumer, L. (1979). Interactions among estrogen, prolactin and luteinizing hormone at the level of adenylyl cyclase in the corpus luteum. Findings and physiological correlates. In: *Proceedings of the 1978 Workshop on Ovarian, Follicular and Corpus Luteum Functions*. (C. P. Channing, I. M. Merth and W. D. Sadler, eds.). Raven Press, New York. pp. 663-677.
- Day, S. L. and Birnbaumer, L. (1980). The effect of estradiol on hormonally stimutable adenylyl cyclase activity and on progesterone production in normal and regressing corpora lutea from control and hCG-treated pseudopregnant rabbits. *Endocrinology* 106, 375-381.
- Demers, L. M., Behrman, H. R. and Greep, R. O. (1973). Effects of prostaglandins and gonadotropins on luteal prostaglandin and steroid biosynthesis. *Adv. Biosci.* 2, 701-707.
- Duddleson, W. G., Midgely, A. R., Jr. and Niswender, G. D. (1972). Computer program sequence for analysis and summary of radioimmunoassay data. *Comput. Biomed. Res.* 5, 205-217.
- Dunn, M. V., Humphries, N. G., Judkins, G. R., Kendall, J. Z. and Knight, G. W. (1973). The effect of prostaglandin F_{2α} antibody on gestation length in the rat. *Prostaglandins*, 3, 509-514.
- Fletcher, P. W., Niswender, G. D. and Reichert, L. E., Jr. (1980). Regulation of receptors for LH and desensitization of ovine luteal adenylate cyclase by homologous hormone. Abstracts of the 62nd Annual Meeting of the Endocrine Society, Washington, D.C. p. 208, Abstr. 536.
- Gibori, G., Antczak, E. and Rothchild, I. (1977). The

- role of estrogen in the regulation of luteal progesterone secretion in the rat after day 12 of pregnancy. *Endocrinology* 100, 1483-1495.
- Goding, J. R., Cumming, I. A., Charnley, W. A., Brown, J. M., Cain, M. D., Cerini, J. C., Mildred, E. D., Cerini, E. D., Findlay, J. K., O'Shea, J. D. and Pemberton, D. H. (1971-72). Prostaglandin $F_{2\alpha}$, "the" luteolysin in the mammal? *Gynecol. Invest.* 2, 73-97.
- Grinwich, D. L., Ham, E. A., Hichens, M. and Behrman, H. R. (1976). Binding of human chorionic gonadotropin and response of cyclic nucleotides to luteinizing hormone in luteal tissue from rats treated with prostaglandin $F_{2\alpha}$. *Endocrinology* 98, 146-150.
- Grinwich, D. L., Kennedy, T. G. and Armstrong, D. T. (1972). Dissociation of ovulatory and steroidogenic actions of luteinizing hormone in rabbits with indomethacin, an inhibitor of prostaglandin biosynthesis. *Prostaglandins* 1, 89-96.
- Harwood, J. P., Dufau, M. L. and Catt, K. J. (1979). Differing specificities in the desensitization of ovarian adenylate cyclase by epinephrine and human chorionic gonadotropin. *Mol. Pharmacol.* 15, 429-444.
- Harwood, J. P., Richert, N. D., Dufau, M. L. and Catt, K. J. (1980). Gonadotropin-induced desensitization of epinephrine action in the luteinized rat ovary. *Endocrinology* 107, 280-293.
- Hichens, M., Grinwich, D. L. and Behrman, H. R. (1974). $PGF_{2\alpha}$ -induced loss of corpus luteum gonadotropin receptors. *Prostaglandins* 7, 449-458.
- Holt, J. A., Keyes, P. L., Brown, J. M. and Miller, J. B. (1975). Premature regression of corpora lutea in pseudopregnant rabbits following the removal of polydimethylsiloxan capsules containing 17β -estradiol. *Endocrinology* 97, 76-82.
- Hunzicker-Dunn, M. and Birnbaumer, L. (1976a). Adenylyl cyclase activities in ovarian tissues. II. Regulation of responsiveness to LH, FSH, and PGE_1 in the rabbit. *Endocrinology* 99, 185-197.
- Hunzicker-Dunn, M. and Birnbaumer, L. (1976b). Adenylyl cyclase activities in ovarian tissues. IV. Gonadotropin-induced desensitization of the luteal adenylyl cyclase throughout pregnancy and pseudopregnancy in the rabbit and the rat. *Endocrinology* 99, 211-222.
- Hunzicker-Dunn, M., Day, S. L., Abramowitz, J. and Birnbaumer, L. (1979). Ovarian responses of pregnant mare serum gonadotropin- and human chorionic gonadotropin-primed rats: Desensitizing, luteolytic and ovulatory effects of a single dose of human chorionic gonadotropin. *Endocrinology* 105, 442-451.
- Keyes, P. L. and Bullock, D. W. (1974). Effects of prostaglandins $F_{2\alpha}$ on ectopic and ovarian corpora lutea of the rabbit. *Biol. Reprod.* 10, 519-525.
- Labhsetwar, A. P. and Watson, D. J. (1974). Temporal relationship between secretory patterns of gonadotropins, estrogens, progestins and prostaglandin-F in periparturient rats. *Biol. Reprod.* 10, 103-110.
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 193, 265-275.
- Marsh, J. (1971). The effect of prostaglandins on the adenylyl cyclase of the bovine corpus luteum. *Ann. N. Y. Acad. Sci.* 180, 416-425.
- McCracken, J. A., Barickowski, B., Carlson, J. C., Green, K. and Samuelsson, B. (1973). The physiological role of prostaglandin $F_{2\alpha}$ in corpus luteum regression. *Adv. Biosci.* 9, 599-624.
- Midgely, A. R., Jr., Niswender, G. D. and Rebar, R. W. (1969). Principles for the assessment of the reliability of radioimmunoassay methods (precision, accuracy, sensitivity, specificity). *Acta Endocrinol. Suppl.* 142, 247-256.
- O'Grady, J., Patrick, B., Caldwell, B. V., Auletta, J. and Speroff, L. (1972). The effects of an inhibitor of prostaglandin synthesis (indomethacin) on ovulation, pregnancy and pseudopregnancy in the rabbit. *Prostaglandins* 1, 97-106.
- Salomon, Y., Londos, C. and Rodbell, M. (1974). A highly sensitive adenylate cyclase assay. *Anal. Biochem.* 58, 541-548.
- Scott, R. S. and Rennie, P.I.C. (1970). Factors controlling the life-span of the corpora lutea in the pseudopregnant rabbit. *J. Reprod. Fertil.* 23, 415-422.
- Shaikh, A. A., Nagvi, R. H. and Saksena, S. K. (1977). Prostaglandins E and F in uterine venous plasma in relation to peripheral plasma levels of progesterone and 20α -hydroxyprogesterone in the rat throughout pregnancy and parturition. *Prostaglandins* 13, 311-320.
- Spies, H. G., Hilliard, J. and Sawyer, C. H. (1968a). Pituitary and uterine factors controlling regression of the corpora lutea in intact and hypophysectomized rabbits. *Endocrinology* 83, 291-299.
- Spies, H. G., Hilliard, J. and Sawyer, C. H. (1968b). Maintenance of corpora lutea and pregnancy in hypophysectomized rabbit. *Endocrinology* 83, 354-367.
- Torjesen, P. A., Dahlin, R., Haug, E. and Aakvaag, A. (1978). The sequence of hormonal changes during prostaglandin-induced luteolysis of the superovulated rat ovary. *Acta Endocrinol.* 87, 617-624.
- Walseth, T. F. and Johnson, R. A. (1979). The enzymatic preparation of [α - ^{32}P] nucleoside triphosphates, cyclic [^{32}P]AMP and cyclic [^{32}P]GMP. *Biochem. Biophys. Acta* 562, 11-31.