STUDIES ON THE MECHANISM OF ACTION OF PROSTAGLANDIN $F_{2\alpha}$ INDUCED LUTEOLYSIS IN RATS.

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ABSTRACT

The effects of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) administration on the utilization of low density lipoprotein (LDL) and progesterone secretion were examined in dispersed luteal cells from rat ovaries. Immature rats were rendered pseudopregnant with administration of pregnant mare serum gonadotropin and human chorionic gonadotropin. Animals were sacrificed at different times after $PGF_{2\alpha}$ (5 mg/kg) or vehicle administration on day-5 of pseudopregnancy. Administration of $PGF_{2\alpha}$ *in vivo* decreased human chorionic gonadotropin (hCG) binding to luteal cell membranes *in vitro* but enhanced binding of LDL. Utilization of labelled cholesterol for steroid synthesis from reconstituted LDL [(^{3}H)-CL-LDL] by dispersed luteal cells was enhanced following $PGF_{2\alpha}$ administration. This suggests that the LDL pathway is not suppressed during prostaglandin induced luteolysis. Progesterone and total progestin secretion in response to N^6-2'-0-Dibutyryladenosine 3'5'-cyclic monophosphate (cAMP) was decreased at 2,4 and 24 hours following $PGF_{2\alpha}$ administration demonstrating a post-cAMP defect in steroidogenesis. Addition of the hydroxylated sterols, 20 or 25-OH cholesterol as substrate stimulated progesterone secretion in vehicle treated rats in a dose dependent fashion with 20-OH cholesterol being more potent. Progesterone secretion in response to stimulation with luteinizing hormone (LH) and cAMP from vehicle treated rats was less than that observed with 20 or 25-OH cholesterol, indicating that endogenous substrate may be a limiting factor in steroid synthesis. The maximal capacity of luteal tissue to produce progestins following $PGF_{2\alpha}$ administration was determined with 20-OH cholesterol as the substrate. The results suggest that the post-cAMP defect at 4 hours following $PGF_{2\alpha}$ administration may be due to failure of the cells to mobilize endogenous cholesterol. However at 24 hours following $PGF_{2\alpha}$ administration the decreased ability of luteal cells to convert cholesterol to pregnenolone may contribute to decreased progesterone synthesis.

INTRODUCTION

$PGF_{2\alpha}$ is the physiological luteolytic factor in a number of animal species including rats (1). Luteal regression has two aspects: structural and functional. Functional luteolysis is characterized in the rat by decreased progesterone and increases in synthesis of 20 α-hydroxy-pregn-4-en-3-one (20-OHP, 2,3). It has been demonstrated that $PGF_{2\alpha}$ inhibits LH - dependent progesterone secretion by the

corpus luteum (4,5). Thomas et al. (6) observed that the inhibitory action of $PGF_{2\alpha}$ on LH-stimulated steroidogenesis in cultured rat luteal cells was reversed by the dibutyryl analog of cAMP. This implies that an important action of $PGF_{2\alpha}$ may be to inhibit cAMP production and that the lesion induced in the steroidogenic pathway beyond cAMP is of lesser significance. The studies of Jordan (7), however, suggest that the $PGF_{2\alpha}$ induced block beyond cAMP production is important in the rat.

The mechanism of $PGF_{2\alpha}$ induced post-cAMP defect in steroidogenesis, is not known. The rate limiting step for all steroidogenic tissues is believed to be the conversion of cholesterol to pregnenolone (8). Torjesen and Aakvaag (9) have demonstrated that progesterone synthesis by the mitochondrial fraction of luteal cells was reduced following $PGF_{2\alpha}$ induced luteolysis. However it is not known whether reduced substrate (cholesterol) or loss of side chain cleavage activity is responsible for decreased steroid synthesis.

Studies have demonstrated that administration of $PGF_{2\alpha}$ reduces the number of LH receptor sites on luteal cell membranes (10,11). Activation of luteal cells by LH is associated with the clustering of LH receptors on the plasma membrane and $PGF_{2\alpha}$ inhibits this response (12). X-ray diffraction studies have suggested that $PGF_{2\alpha}$ induced luteal regression involves phase changes in the phospholipid bilayer of the cell membrane resulting in decreased gonadotropin binding (13-15).

$PGF_{2\alpha}$ induced changes in luteal cells include reduction in the storage of cholesterol esters (16). It is well established that cholesterol is imported into the luteal cell in the form of a lipoprotein complex by a membrane receptor mediated process. Rat luteal cells can utilize cholesterol carried by high density lipoprotein (HDL) or LDL as substrate in steroidogenesis (17, 18). The strategies used for delivery of lipoprotein transported cholesterol to steroid secreting cells are strikingly different for HDL and LDL. HDL is internalized following binding but delivers cholesterol to luteal cells without undergoing complete degradation (19). In contrast, the LDL pathway involves the endocytosis of intact LDL followed by lysosomal degradation of the lipoprotein to liberate cholesterol (17, 18). Our preliminary observations suggest that administration of $PGF_{2\alpha}$ does not reduce high density lipoprotein binding to rat luteal membranes (20). No information is available concerning LDL binding or internalization and the consequent availability of the substrate for steroidogenesis.

The present studies were carried out to compare the effects of $PGF_{2\alpha}$ on the membrane binding of LH and LDL and also to determine its effects on utilization of LDL borne cholesterol for steroid synthesis. In addition, we have examined whether decreased availability of cholesterol to the mitochondrial cholesterol side chain cleavage system is responsible for decreased progesterone secretion during $PGF_{2\alpha}$ induced luteolysis.

MATERIALS AND METHODS

1. Treatment and luteal cell incubations.

1.1 $PGF_{2\alpha}$ treatment.

Immature female Sprague-Dawley rats (21 to 23 days old) were rendered pseudopregnant by the subcutaneous injection of 50 IU of Pregnant Mare Serum Gonadotropin (Ayerst laboratories, Montreal) followed 60 hours later by an injection of 100 IU of hCG (Sigma, St.Louis, MO.) Four days later, $PGF_{2\alpha}$ was administered in the form of Dinoprost tromethamine (Lutalyse, Upjohn, Orangeville, Ontario) at a dosage of 5 mg/kg. The rats were decapitated at 0, 2, 4 or 24 h after $PGF_{2\alpha}$ administration on day 5 of pseudopregnancy.

1.2 Cell dispersion and incubation

The ovarian cells from these superluteinized ovaries were dispersed with collagenase as described (21). Cell viability was determined by trypan blue exclusion. After one hour preincubation, 0.5 to 1.0 x 10^6 cells were washed and incubated for 4 hours in 1 ml of (Eagle's) Minimum essential medium (MEM, Gibco, Grand Island, NY) at 37°C with graded doses of o-LH, dibutyryl cAMP (Sigma, St. Louis, MO), 25-OH cholesterol (Steraloids, Wilton, NH.) and 20-OH cholesterol (Sigma, St. Louis, MO). To determine the effect of aminoglutethimide, an inhibitor of cholesterol side-chain cleavage, on 20-OH cholesterol induced progesterone secretion, luteal cells were pre-incubated with aminoglutethimide (1 mM) for 30 minutes prior to addition of 20-OH cholesterol. At the end of the incubation the cells and medium were separated by centrifugation at 200 x g. The cell pellet was then washed with normal saline and dissolved in 0.5 N NaOH for protein estimation. Progesterone and 20-OHP levels in the media were measured by radioimmunoassay (21, 22).

2. Binding of ^{125}I-LDL to luteal cell membranes.

2.1 Isolation of LDL

Human lipoprotein fractions were isolated from blood plasma collected in 0.1% EDTA from healthy donors. LDL (density, 1.019-1.063 g/ml) was isolated in the ultracentrifuge by differential density flotation, using KBr for density adjustments (23). Further purification was achieved as described elsewhere (18). Isolated LDL was dialyzed for 48 hours against four changes of 0.15M NaCl containing 0.3 mM EDTA.

2.2 Iodination of LDL and saturation analysis.

Human LDL was iodinated according to the iodine monochloride method of McFarlane (24) and was extensively dialyzed against phosphate buffered saline (PBS) containing 0.3 mM EDTA. Iodinated lipoprotein was more than 98% precipitable with trichloracetic acid. The specific activity of ^{125}I-LDL was 350 cpm/ng protein. Luteal cells from pseudopregnant rats were incubated in MEM containing 1% bovine

serum albumin (BSA, Sigma, St. Louis, MO.) with graded doses of labelled LDL (1 to 300 ug). After a two hour incubation at 37°C, the cells were washed three times with 2.5 ml of ice cold MEM. After washing, the cells were incubated for 60 minutes at 4°C in ice cold MEM with or without 10 mg/ml of heparin (Sigma, St. Louis, MO.). At the end of the incubation the cells were centrifuged at 200 x g and the amount of radioactivity released into the media was determined. The difference between the radioactivity released in the presence or absence of heparin was considered to be heparin releasable or surface bound LDL (25).

2.3 $PGF_{2\alpha}$ treatment on iodo LDL binding.

Luteal cells obtained at 0, 4 & 24 hours after $PGF_{2\alpha}$ treatment were incubated in MEM containing 1% BSA with a saturating dose of ^{125}I-LDL (100 ug). After two hours of incubation the heparin-releasable LDL was determined as described earlier. Heparin-releasable LDL from luteal cell membranes was expressed as ug/mg protein.

3. LH receptor binding assays.

3.1 Preparation of membrane fraction.

Ovaries were removed from rats at 2, 8, 16 and 24 h after $PGF_{2\alpha}$ or vehicle treatment. The ovaries were then trimmed of connective tissue, minced and homogenized with ice-cold 0.25M sucrose solution. All steps involving the membrane fraction were performed at 4°C. The homogenate was centrifuged at 600 x g for 10 minutes, the supernatant was separated and centrifuged at 12,000 x g for 20 minutes. These partially purified membrane fractions were stored at -70°C until assayed for hCG binding activity as an indication of LH receptor number.

3.2 Membrane incubation.

Purified hCG (CR-121) was iodinated by the lactoperoxidase technique (26). Binding to ovarian membranes was determined by incubating 100 ug of membrane fraction with 7 ng of ^{125}I-hCG (45,000 CPM). The non-specific binding was estimated by the addition of 1 ug non labelled hCG. The buffer used in the binding assay was 50 mM Tris-HCl buffer (pH-7.4) containing 5 mM $MgSO_4$ and 1% BSA. The tubes were incubated for 16 hours at room temperature on a rotary shaker. At the end of the incubation the tubes were centrifuged at 12,000 x g for 20 minutes at 4°C. The membrane fraction was washed once with binding assay buffer without BSA and the radioactivity associated with the membrane was counted in a Beckman gamma 8000 counter. The membrane pellets were then dissolved in 0.5N NaOH for protein determination by the method of Lowry et al. (27). The non-specific binding was subtracted from the total binding to obtain an estimate of the amount of hCG that was specifically bound to receptors.

4. Incubation of luteal cells with reconstituted LDL.

4.1 Preparation of reconstituted (^{3}H) CL-LDL.

The (^{3}H) cholesteryl linoleate was prepared as described by Goodman (28). Cholesteryl esters from LDL were exchanged with (^{3}H) cholesteryl linoleate and reconstituted as described previously by Kreiger et al. (29). Purity of the reconstituted LDL was assessed by mixing it with fresh plasma and subjecting it to differential density flotation.

4.2 Incubation and extraction of steroids.

One million luteal cells from vehicle or $PGF_{2\alpha}$ treated rats were incubated in MEM containing 100 μg (^{3}H)CL-LDL for 4 hours at 37°C. Following incubation, the medium and cells were separated by centrifugation. 20 μg of each of the non-labelled steroids (pregnenolone, progesterone, 20-OHP and cholesterol) were added to the media and were extracted three times with petroleum ether. Our preliminary studies indicated that the recovery of ^{3}H-progesterone from media with petroleum ether extraction ranged between 81 to 85%. The extracted samples were then evaporated to dryness in a water bath under a stream of nitrogen. The residue was redissolved in chloroform-methanol 1:1 mixture and spotted onto 20x20 cm glass chromatography plates coated with silica gel GF (BDH, Toronto, Ontario). The thickness of the coating was approximately 250 μ. Two dimensional thin-layer chromatography was performed using chloroform-dioxane (96:4) as the mobile phase in the first dimension and ethyl acetate-dioxane (96:5) in the second dimension. Steroids were located by UV light and iodine vapour. The areas of chromatograph, like authentic pregnenolone, progesterone, 20-OHP and cholesterol were then scraped separately, eluted with 10 ml of ethyl acetate, evaporated to dryness and the amount of ^{3}H incorporated into each steroid was determined by counting in a Packard beta scintillation counter.

Statistical Analysis

Data obtained from incubation experiments were subjected to nested analysis of variance. When a significant F value was present Duncan's new multiple range test was used for individual comparisons between treatment means. Some individual comparisons between means were made by Students t-test.

RESULTS

1. Effect of $PGF_{2\alpha}$ treatment on progesterone accumulation

The effects of $PGF_{2\alpha}$ treatment on progesterone accumulation by rat luteal cells in response to graded doses of LH and cAMP is depicted in figure-1. Analysis of variance revealed progesterone accumulation by luteal cells collected 2 hours after $PGF_{2\alpha}$ treatment was significantly lower compared to the vehicle treated group ($p<0.01$). LH and cAMP enhanced progesterone accumulation in both vehicle and

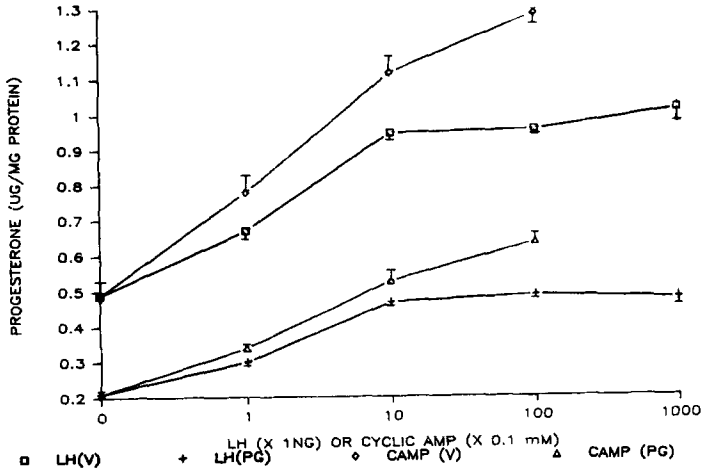

Figure-1 Effect of varying concentrations of LH and cAMP on progesterone in the medium after incubating rat luteal cells collected at 2 hours after $PGF_{2\alpha}$ (PG) or vehicle (V) administration in vivo. Luteal cells were incubated with graded doses of LH or cAMP for 4 hours at 37°C in an atmosphere of 95% O_2 and 5% CO_2. At the end of incubation the medium was separated from cells for progesterone estimation. All values are Mean ± SEM (n=6).

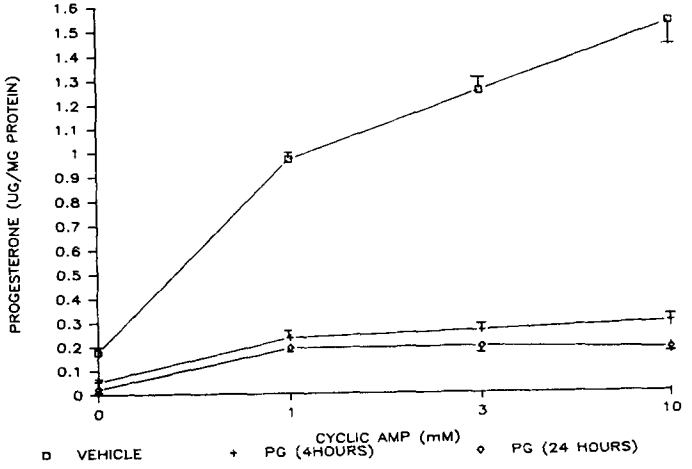

Figure-2 Effect of cAMP on progesterone accumulation by luteal cells obtained at 4 and 24 hours following $PGF_{2\alpha}$ administration. Luteal cells were incubated under conditions as described in figure legend-1. All values are Mean ± SEM (n=6).

$PGF_{2\alpha}$ treated groups ($p<0.01$). Maximal progesterone accumulation following LH or cAMP was lower in the $PGF_{2\alpha}$ treated group ($p<0.01$). The stimulatory effect of cAMP on progesterone accumulation in medium by luteal cells was also suppressed at 4 and 24 hours after $PGF_{2\alpha}$ treatment (figure-2). Addition of cAMP enhanced 20-OHP accumulation in luteal cells from both $PGF_{2\alpha}$ and vehicle treated rats in a dose dependent fashion (figure-3). The accumulation of 20-OHP in response to cAMP was reduced 24 hours following $PGF_{2\alpha}$ administration ($p<0.001$). The total progestin accumulation by luteal cells decreased with time following $PGF_{2\alpha}$ treatment. Addition of cAMP to luteal cells from the $PGF_{2\alpha}$ treated group failed to reach the progestin maxima observed in luteal cells from vehicle treated control animals ($p<0.01$). The ratio of 20-OHP/progesterone was enhanced at 4 and 24 hours of $PGF_{2\alpha}$ treatment compared to the vehicle treated group ($p<0.01$, figure-4).

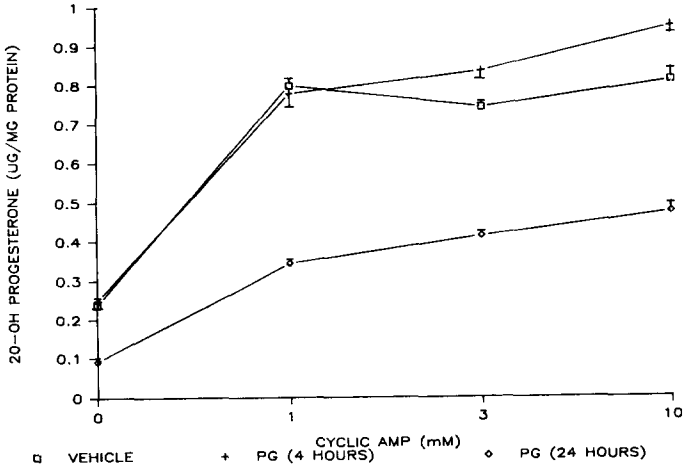

Figure-3 Effect of cAMP on 20-OHP accumulation by luteal cells obtained after administration of $PGF_{2\alpha}$ at time periods as indicated in the figure. Luteal cells were incubated under conditions as described in figure legend-1. All values are Mean ± SEM (n=6).

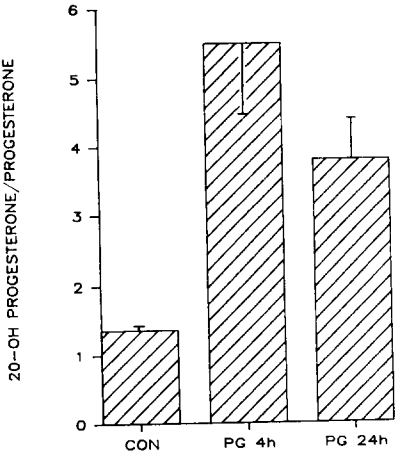

Figure-4 Ratio of 20-OHP/progesterone following $PGF_{2\alpha}$ administration. Luteal cells were obtained at different times after $PGF_{2\alpha}$ administration and were incubated as described in figure legend-1. At the end of 4 and 24 hours, progesterone and 20-OHP levels were determined (n=5).

2. Effect of exogenous substrate on progesterone accumulation by luteal cells

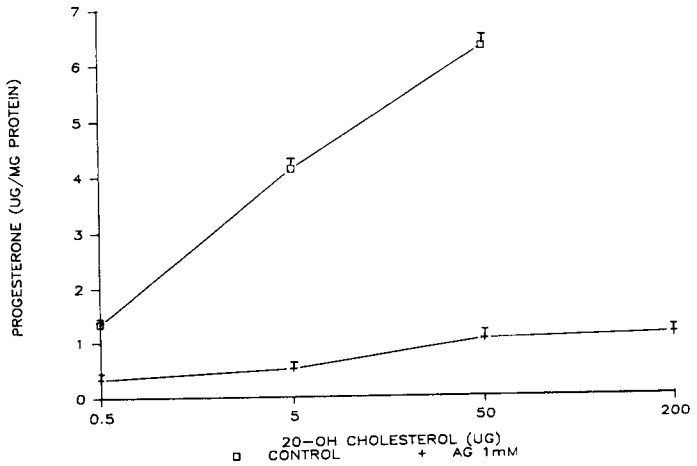

Figure-5 Effect of aminoglutethimide (AG) on 20-OH cholesterol induced progesterone stimulation. Luteal cells from day-5 pseudopregnant rats were incubated for 4 hours at 37oC with varying doses of 20-OH cholesterol in the presence or absence of AG (1mM). All values are Mean ± SEM (n=5).

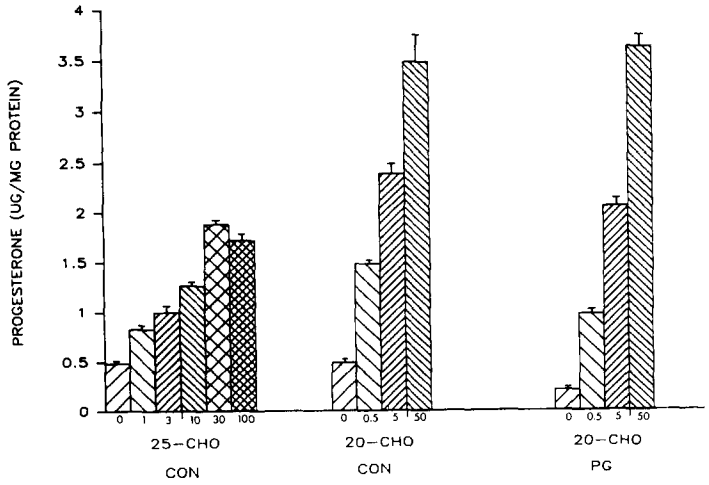

Figure-6 Effect of varying concentration of 25 or 20-OH cholesterol ($\mu g/ml$) as substrate on progesterone accumulation by luteal cells obtained either before or 2 hours following $PGF_{2\alpha}$ administration. All values are Mean $\pm$ SEM (n=6). Control group is represented as CON.

Incubation of luteal cells from the vehicle treated group with 20-OH cholesterol augumented progesterone accumulation ($p<0.01$, Figure-5). Addition of aminoglutethimide (1mM) significantly inhibited ($p<0.01$) 20-OH cholesterol induced progesterone accumulation (figure-5). Comparison of means indicated that progesterone accumulation in presence of 20-OH cholesterol was significantly greater than when

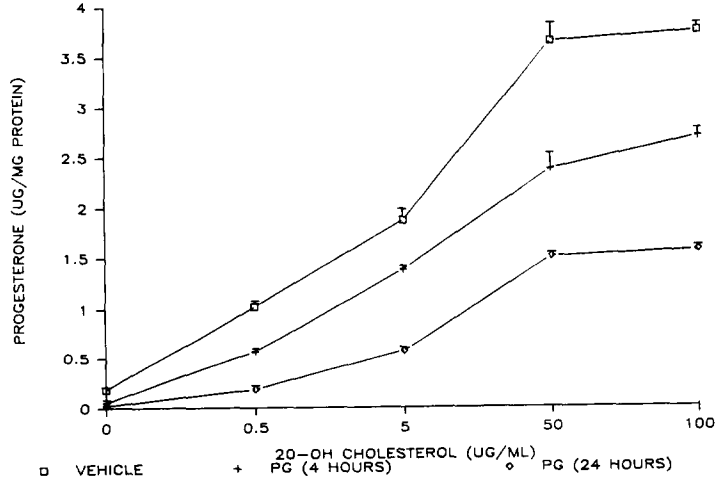

Figure-7 Progesterone accumulation by luteal cells in response to varying concentrations of 20-OH cholesterol. Luteal cells obtained at 0, 4 and 24 hours after $PGF_{2\alpha}$ administration were incubated for four hours at 37°C with 20-OH cholesterol. All values are Mean $\pm$ SEM (n=6).

cells were provided with 25-OH cholesterol as substrate ($p<0.01$, figure-6). In cultures of luteal cells obtained 2 hours following treatment of $PGF_{2\alpha}$, progesterone accumulation was significantly (50 μg) lower ($P<0.01$) than in the vehicle treated group. Addition of 20-OH cholesterol as substrate was able to overcome $PGF_{2\alpha}$ induced suppression at 2 h (figure-6). Four hours following $PGF_{2\alpha}$ treatment progesterone accumulation by luteal cells in response to 20-OH cholesterol substrate was significantly lower ($p<0.01$) in the $PGF_{2\alpha}$ treated group as compared to the vehicle treated control (figure-7). Progesterone accumulation in the medium was further reduced in luteal cell cultures prepared from rats killed 24 hours after $PGF_{2\alpha}$ treatment (figure-7). The effect of 20-OH cholesterol as substrate for 20-OHP secretion by luteal cells following treatment with $PGF_{2\alpha}$ is depicted in figure-8. The levels of 20-OHP in response to addition of substrate were significantly higher in culture medium of luteal cells from $PGF_{2\alpha}$ treated group (4 hours) as compared to vehicle treatment ($p<0.01$, figure-8). However accumulation of 20-OHP was significantly reduced after 24 hours exposure in vivo following $PGF_{2\alpha}$ administration compared to 4 hour post treatment group ($p<0.01$, Fig-8). Total progestin secretion in response to 20-OH cholesterol was significantly greater ($p<0.01$) in luteal cells taken 4 hours after $PGF_{2\alpha}$ treatment compared to vehicle treated group. However, the accumulation of total progestins in response to 20-OH cholesterol was lower following 24 hour of $PGF_{2\alpha}$ treatment ($p<0.01$).

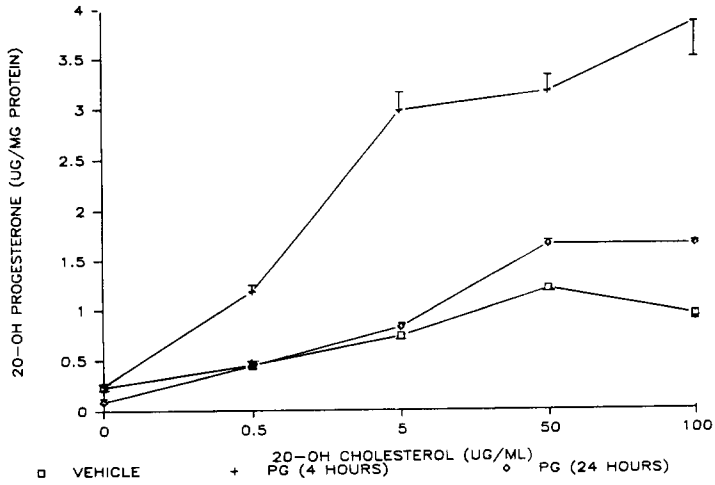

Figure-8 Luteal cell secretion of 20-OHP in response to 20-OH cholesterol. Experimental details are as described in figure legend-8. (Mean ± SEM, n=6).

3. Saturation analysis for heparin releasable LDL.

Incubation of luteal cells with heparin (10 mg/ml) released membrane bound ^{125}I-LDL. Statistically definable ($p<0.01$) heparin

release was observed with 3 μg of ^{125}I-LDL. The release of LDL plateaued between 30 and 300 μg. The slight increase in heparin releasable LDL with 300 μg was not statistically different from that observed with 100 ug (figure-9).

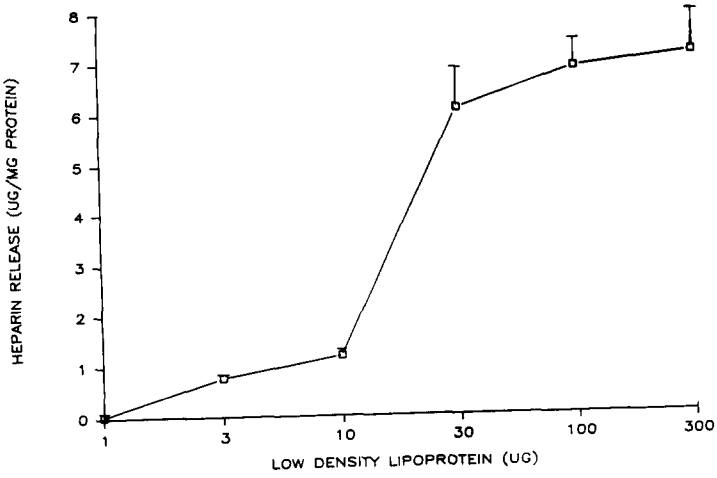

Figure-9 Saturation analysis of heparin releasable ^{125}I-LDL. Luteal cells from rats were incubated at 37°C for 2 hours with graded doses of ^{125}I-LDL. The cells were then washed twice and incubated in MEM with or without 10 mg/ml heparin. Heparin releasable radioactivity was determined and expressed as μg of lipoprotein released/mg protein. All values are mean $\pm$ SEM (n=5).

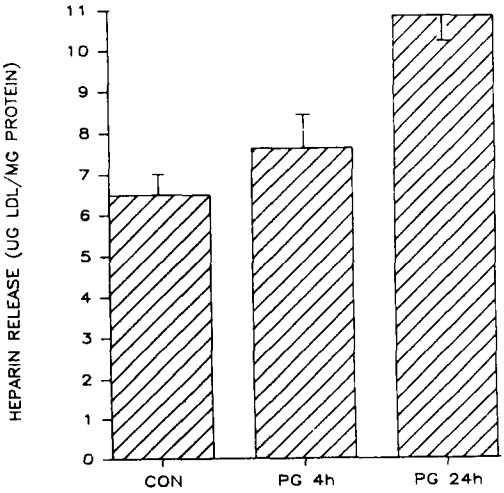

Figure-10 Effect of $PGF_{2\alpha}$ treatment on heparin releasable LDL. Luteal cells at different time periods of $PGF_{2\alpha}$ administration were incubated with 100 ug/ml of ^{125}I-LDL for 2 hours. Heparin releasable LDL was determined as described in figure legend-9.

4. $PGF_{2\alpha}$ pretreatment on heparin releasable LDL from luteal cells.

One way analysis of variance indicated that $PGF_{2\alpha}$ treatment significantly ($p<0.01$) influenced the quantity of LDL released by heparin in a time dependent fashion (figure-10). The heparin releasable LDL was significantly greater ($p<0.01$) at 24 hour after $PGF_{2\alpha}$ treatment as compared to vehicle treated control.

5. UTILIZATION OF RECONSTITUTED LDL

Pregnenolone and 20-OHP rose steadily with time after administration of $PGF_{2\alpha}$. The increase in 20-OHP with $PGF_{2\alpha}$ administration was statistically significant ($p<0.05$, figure-11). There was no significant change in pregnenolone. Progesterone levels at 4 and 24 hours was not significantly different from vehicle treated group. The total secretion of pregnenolone + progesterone + 20-OHP did not change at 4 hours after $PGF_{2\alpha}$ administration but was significantly enhanced after 24 hours of treatment ($p<0.05$, figure-11).

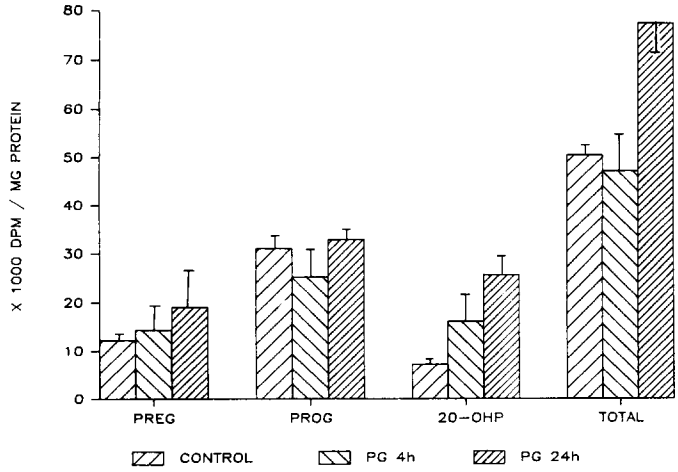

Figure-11 Utilization of reconstituted LDL. Luteal cells obtained from pseudopregnant rats at different times after $PGF_{2\alpha}$ administration were incubated with (^{3}H)CL-LDL for 4 hours. At the end of incubation steroids were extracted and separated by thin layer chromatography. Incorporation of labelled cholesterol into steroids were quantitated. All values are Mean $\pm$ SEM (n=5).

6. Effect of $PGF_{2\alpha}$ treatment on serum progesterone and hCG binding.

The time course of the effect of $PGF_{2\alpha}$ pretreatment on serum progesterone and hCG binding to luteal cell membrane is shown in Table-1. A reduction in circulating progesterone was observed within 2 hours of $PGF_{2\alpha}$ treatment ($p<0.01$). Similarly, reduced progesterone levels were present 8 hours through 24 hours. Binding of hCG did not significantly change during the first 8 hours of $PGF_{2\alpha}$ administration.

A significant decrease in hCG binding was observed at 16 and 24 hours after $PGF_{2\alpha}$ administration ($p<0.01$).

Table-1

Time course effect of $PGF_{2\alpha}$ on serum progesterone and hCG binding to the luteal cell membrane.

	Prog ng/ml serum Mean ± SEM	hCG binding 10^5 cpm/mg protein Mean ± SEM
0h	337.7 ± 52.55 (n=17)	3.47 ± 0.22 (n=18)
2h	106.87 ± 35.36 (n=5)	4.06 ± 0.5 (n=4)
8h	121.29 ± 12.45 (n=5)	3.16 ± 0.49 (n=4)
16h	104.97 ± 24.71 (n=7)	1.73 ± 0.44 (n=7)
24h	87.61 ± 15.6 (n=5)	2.06 ± 0.29 (n=6)

DISCUSSION

In the present study we have presented data to support that $PGF_{2\alpha}$ produces luteolysis, including the expected decline in circulating progesterone followed by a reduction in luteal membrane gonadotropin binding. This sequence concurs with the findings of Grinwich et al. (10) and suggests that the initial progesterone reduction in luteolysis occurs by a mechanism other than the reduction in luteotrophic support.

There is also ample support in the present study for a negative effect of $PGF_{2\alpha}$ on luteotrophic stimulation of rat luteal cells. Administration of $PGF_{2\alpha}$ in vivo inhibited the capacity of luteal cells to respond to LH in vitro, a finding which concurs with earlier reports (7,9). Total progesterone accumulation in vitro in response to cAMP was also reduced following treatment with $PGF_{2\alpha}$ in vivo. This suggests impairment of luteotrophic stimulation beyond the accumulation of cAMP in agreement with the same recent reports (30, 31) and in contrast to others (32).

It is of interest to note that the luteolytic effects appear not to involve the process of LDL utilization by the rat luteal cells. In the present study, membrane associated LDL was determined by the method of Goldstein et al. (25), in which LDL which has not been

method of Goldstein et al. (25), in which LDL which has not been internalized can be released by treatment with the polyanion heparin. The results suggest that $PGF_{2\alpha}$ treatment increases the surface binding of LDL. This finding is perplexing from two points of view. First, it has previously been shown that treatment with gonadotropins increases lipoprotein binding sites in rat luteal membranes (33). Evidence from the present study suggests that $PGF_{2\alpha}$ interferes with gonadotropin binding and postbinding stimulation of cellular activity. It would therefore be expected to decrease lipoprotein binding. Second, Buhr et al. (13) have suggested that $PGF_{2\alpha}$ alters luteal membranes from the sol to gel state thereby disrupting gonadotrophin binding. The present findings, as well as our previous report that $PGF_{2\alpha}$ treatment effects no reduction in HDL binding sites (20) suggests that, if the proposed membrane disruption occurs, it has no effect on the receptors for lipoproteins.

Intracellular cholesterol has been shown to regulate LDL receptors by a negative feedback mechanism (34). Behrman et al. (16) have shown that administration of $PGF_{2\alpha}$ to rats lowers the luteal levels of cholesterol esterase. The time-dependent increases in surface binding of LDL following $PGF_{2\alpha}$ treatment may be a result of depletion of intracellular cholesterol and consequent amplification of LDL receptor numbers by an increased rate of receptor synthesis, recycling or both.

Incubation of luteal cells taken at various intervals after $PGF_{2\alpha}$ treatment with reconstituted LDL containing ^{3}H-cholesterol provides further support for the view that $PGF_{2\alpha}$ induced luteolysis does not alter lipoprotein utilization by luteal cells. This corroborates the recent findings of Pate and Nephew (35) who have demonstrated that bovine luteal cells, obtained from CL undergoing functional regression following $PGF_{2\alpha}$ administration in vivo, were able to utilize LDL or HDL to enhance steroidogenesis. In the present study luteal cells were capable of synthesizing labelled steroids from the ^{3}H-cholesterol provided in the form of LDL. There were quantitative differences in the labelled steroids synthesized as luteolysis progressed. The elevation in 20-OHP at the expense of progesterone is in keeping with the finding of Grinwich et al. (10). It is of interest to note that total labelled steroid production increased at 24 h after $PGF_{2\alpha}$ treatment. This may result from an augmentation in LDL utilized as suggested by the increases in surface binding of LDL described above.

Toaff et al. (36) reported that progesterone production by rat luteal cells in vitro was limited by the quantities of endogenous cholesterol available for substrate. They further suggested that maximal steroidogenic potential of luteal cells in vitro can be studied by the addition of exogenous substrate in the form of hydroxylated cholesterol which bypasses the supposed rate limiting process of sterol movement to the side-chain cleavage enzyme. Luteal cells derived from control (vehicle treated) rats in the present study produced more progesterone when incubated with 20-OH or 25-OH cholesterol than cells incubated with maximal doses of LH or cAMP. This is in agreement with the concept of substrate limitation in luteal cells of Toaff et al. (36).

The capability of luteal cells taken at various intervals after $PGF_{2\alpha}$ to produce progestins in vitro following addition of 20-OH cholesterol was evaluated in the present study. No reduction in progesterone production in the presence of hydroxylated cholesterol was noted at 2 h after $PGF_{2\alpha}$ in spite of diminuition of the cAMP stimulated progesterone accumulation at this time. This finding suggests that there was no impairment of the mitochondrial enzyme complex responsible for cleavage of the cholesterol to pregnenolone (37). At 4 h after $PGF_{2\alpha}$, less progesterone accumulated over the incubation period when 20-OH cholesterol was present. However, as has been described in vivo, the decrease in progesterone was associated with an increase in 20-OHP (10). The sum of these progestins was greater at four hours after $PGF_{2\alpha}$. We therefore conclude that no impairment of the cholesterol cleavage enzyme occurs in the first 4 h following treatment with a luteolytic dose of $PGF_{2\alpha}$ in vivo.

By 24 h after $PGF_{2\alpha}$, there was significant loss in the capacity of luteal cells to produce progestins in the presence of exogenous 20-OH cholesterol. This suggests that a lesion in the cholesterol side chain cleavage enzyme complex is one of the late events in $PGF_{2\alpha}$ induced luteolysis. It is puzzling that total steroid production from LDL associated ^{3}H-cholesterol increased at 24 h after $PGF_{2\alpha}$. An explanation for this phenomenon requires further experimentation.

In summary, the present study suggests that the pathway for LDL utilization by rat luteal cells is unaffected during $PGF_{2\alpha}$ induced luteolysis. Reductions in progesterone secretion at 2 and 4 h after $PGF_{2\alpha}$ treatment may be due to changes in the availability of endogenous cholesterol to the side-chain cleavage enzyme. At 24 h after $PGF_{2\alpha}$ administration the decline in progesterone synthesis may be attributable to decreased activity of the mitochondrial cholesterol side chain cleavage enzyme system.

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