Control of progesterone production in small and large bovine luteal cells separated by flow cytometry

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Summary. Corpora lutea were collected from Holstein heifers on Days 10 and 12 of the oestrous cycle and the cells were dispersed with collagenase. The dispersed cells were separated into preparations of highly purified (90–99%) small (<20 µm) and large (>25 µm) luteal cells by unit gravity sedimentation and fluorescence-activated cell sorting. Net progesterone accumulation by 1×10^5 small cells and 1×10^3 large cells during 2 and 4 h incubations, respectively, were measured after additions of LH, PGF-2α, and phorbol esters, alone and in combination. Progesterone synthesis was increased ($P < 0.05$) by phorbol dibutyrate (PBT₂) or PGF-2α ($P < 0.05$) in small, but not in large, luteal cells (10.1 ± 3.0 and 18.1 ± 5.0 ng/10⁵ cells for 0 and 50 nM-PBT₂, and 19.9 ± 3.2 and 44.2 ± 9.3 ng/10⁵ cells for 0 and 1 µg PGF-2α/ml). The previously reported stimulatory effects of PKC activation and PGF-2α addition to total dispersed cell preparations are therefore entirely attributable to the small, theca-derived cells. Small cells responded to low levels of LH (9.1 ± 1.1 , 69.0 ± 5.4 and 154.7 ± 41.4 ng/10⁵ cells for 0, 1 and 5 ng LH/ml, respectively, $P < 0.05$), while large cells responded only to high levels of LH (1635 ± 318 , 2662 ± 459 and 3386 ± 335 pg/10³ cells for 0, 100 and 1000 ng LH/ml, respectively, $P < 0.05$). PGF-2α inhibited LH-, 8-Br-cAMP- and forskolin-stimulated progesterone synthesis in the large cells (3052 ± 380 , 3498 ± 418 , 3202 ± 391 pg/10³ cells for 1 µg LH/ml, and 0.5 mM-8-Br-cAMP, and 1 µM-forskolin respectively and 1750 ± 487 , 2255 ± 468 , 2165 ± 442 pg/10³ cells for PGF-2α + LH, PGF-2α + 8-Br-cAMP and PGF-2α + forskolin, respectively), indicating that the inhibitory effect of PGF-2α on progesterone synthesis in large cells occurs at a site distal to cAMP generation. These results suggest that the large cells are the targets of the luteolytic effects of PGF-2α, while the small cells are responsible for the previously reported luteotrophic effect of PGF-2α *in vitro*.

Keywords: corpus luteum; cell sorter; prostaglandin-F2α; progesterone; cow

Introduction

In recent studies it has been demonstrated that mature cow (Ursely & Leymarie, 1979; Koos & Hansel, 1981), sheep (Fitz *et al.*, 1982), pig (Lemon & Loir, 1977) and rabbit (Hoyer *et al.*, 1986) luteal tissues consist of at least two morphologically and biochemically distinct cell types that can be separated according to size and differ in their ability to secrete progesterone. In the bovine corpus luteum (CL), preparations of the small cells (10–22 µm in diameter) are about 6 times more responsive to luteinizing hormone (LH) added *in vitro* than are those of the relatively impure large cells (>25 µm) (Ursely & Leymarie, 1979; Koos & Hansel, 1981). In sheep the large cells produced more progesterone than the small cells but were unresponsive to LH, dibutyryl cAMP, or agents that stimulate cAMP such as cholera toxin and forskolin (Fitz *et al.*, 1982; Hoyer *et al.*, 1984). In

the cow CL, the large cells were reported to be marginally responsive to added LH (Ursely & Leymarie, 1979; Koos & Hansel, 1981) and it was suggested that the observed responses to LH were due to contaminating small cells in the preparations. These observations suggested that steroidogenesis in these cell types is regulated by different mechanisms, and this may be important in the regulation of the functional lifespan of the CL.

In earlier experiments with enzyme-dispersed total luteal cell preparations, Hansel & Dowd (1986) and Brunswig *et al.* (1986) showed that addition of phorbol esters increased progesterone production. Addition of a phorbol ester that does not activate protein kinase C failed to increase progesterone production (Hansel *et al.*, 1987). Dowd & Hansel (1987) further substantiated that the stimulatory effect of addition of a phorbol ester on progesterone production by bovine luteal cells is due to protein kinase C activation by showing that protein kinase C enzyme activity and specific binding to phorbol ester co-eluted as a single peak when luteal cell cytosolic fractions were chromatographed on DEAE-Sephadex. In total dispersed cell preparations, PGF-2 α , like phorbol esters, stimulates progesterone production by bovine luteal cells (Hixon & Hansel, 1979) by a mechanism that involves the Ca²⁺-polyphosphoinositol protein kinase C (Ca²⁺-PKC) system (Davis *et al.*, 1987a, b).

Work with the large luteal cells has been hampered by lack of a good separation system. Separation methods such as unit gravity sedimentation (Koos & Hansel, 1981) or elutriation (Fitz *et al.*, 1982) have yielded large cell preparations that are usually contaminated with large numbers of small cells. Production of pure large cell preparations by micromanipulation (Hansel *et al.*, 1987) is slow and tedious. In the present work, we used flow cytometry and unit gravity sedimentation to obtain pure preparations of large and small luteal cells from bovine CL; this development enabled us to compare the influences of various substances on large and small cells from the same corpora lutea.

Materials and Methods

Tissue dissociation and cell separations. Corpora lutea (CL) were enucleated from normal Holstein heifers on Days 10–12 of the oestrous cycle (oestrus = Day 0) and immediately placed in cold (4°C) Medium 199 containing penicillin–streptomycin (100 units penicillin/ml and 100 μ g streptomycin/ml). Dispersed cell preparations were made and viable cells were determined by counting cells that excluded Trypan blue (Simmons *et al.*, 1976).

Briefly, the luteal tissue was dissociated in collagenase (2000 U/g tissue) in Medium 199 (5 ml/g luteal tissue) at 34°C. After incubation for 45 min, the medium containing cell suspension was decanted, centrifuged (100 g, 5 min), and the cells were resuspended in fresh medium. Fresh medium containing collagenase was added to the remaining undissociated tissue and dissociation was continued for an additional 45 min. The cells from this dissociation were also centrifuged, and cells from both dissociations were pelleted. Centrifugation was repeated three times, and the cells were resuspended in cold fresh medium after each centrifugation. The cells were filtered through an artist's silkscreen (150 μ m pore diameter) followed by a monofilament nylon mesh (44 μ m) to remove clumps of undissociated tissue. Cells were separated by unit gravity sedimentation (Koos & Hansel, 1981) which yielded preparations that were 95–100% small cells. These fractions of small cells were free of large cells but slightly contaminated with endothelial cells. The fraction containing large cells was collected and sorted in order to obtain pure fractions of large cells. Purified small cells were also used during the analysis by flow cytometry to help identify the window containing the large cells and to determine the effects, if any, of the laser beam on cell viability.

Flow cytometry. Flow analysis and sorting of the luteal cells was carried out on a BD FACS 440 (Becton Dickinson, Mountain View, CA) using an argon ion laser (Spectra Physics model 164-05, Mountain View, CA) with Medium 199 as sheath fluid. The sheath pressure was 96.5 kPa and the injector tip was 70 μ m in diameter. Cells were excited with the 488 nm line at approximately 150 mW output. Relative autofluorescence was measured at 578 nm using a 550 nm longpass filter in conjunction with a 600 nm shortpass filter (both from Corion Corp., Holliston, MA), while forward low-angle scatter was determined using a 488/10 nm bandpass filter and a 1.6 optical density neutral filter (Becton Dickinson).

A computer (Digital Electronics Corp. PDP 11/23) interfaced with the FACS was used to compute the 2-dimensional distribution of any test population of cells with respect to forward low-angle scatter and the log of the relative autofluorescence. The subsequent cytograms were displayed on an associated visual display unit with relative autofluorescence measurements being expressed as log units on the ordinate, and forward low-angle scatter measurements as linear units on the abscissa. Sort rates for experiments were 400–600 cells/sec. The sorting windows were characterized such that cells with high relative autofluorescence were isolated and cell clumps, small luteal cells, and

cells less than 10 μm in diameter were excluded. The purity and viability of sorted cells were determined by light microscopy on the basis of size. This method of classification of the cells has been validated by electron microscopy to identify each cell type (Koos & Hansel, 1981; Hansel *et al.*, 1987; Hansel & Dowd, 1986).

Incubations and progesterone assay. Treatments (10 μl) were added (0 h) and aliquants were removed after 10 min preincubation. Incubations were conducted with 1×10^5 viable small luteal cells per 0.5 ml Medium 199 for 2 h while 1×10^3 viable large luteal cells were incubated for 4 h. Samples were collected and frozen at -20°C until assayed for progesterone. There were 3 or 4 replicates of each treatment for each CL for large and small cells respectively. Net progesterone accumulation, representing medium plus cells, was the difference between concentrations at 0 and 2 or 4 h.

Progesterone concentrations were determined by radioimmunoassay (Beal *et al.*, 1980). The antiserum used was specific for progesterone, cross-reacting with 5β -dihydroprogesterone (13.6%), 20β -dihydroprogesterone (5.7%), 17α -hydroxyprogesterone (4.4%), 20α -dihydroprogesterone (1.8%), 5α -dihydroprogesterone (1.6%) and corticosterone (1.4%). All other steroids tested cross-reacted $<1\%$. The sensitivity was 10 pg/tube and the intraassay coefficient of variation was 4.5% at 60 pg/tube (18 assays). Interassay coefficients of variation were 8.3% and 7.6% at 60 and 300 pg/tube, respectively. The samples from each CL were analysed in the same assay.

Reagents. Medium 199 and penicillin-streptomycin were purchased from Grand Island Biological Co. (Grand Island, NY). Collagenase (CLS) was obtained from Worthington Biochemicals (Freehold, NJ), 4β -phorbol 12, 13-dibutyrate (PBT_2), forskolin, 8-bromo-adenosine 3,5-cyclic AMP (8-Br-cAMP), and phospholipase C (Type XIII, 2500 Units/mg) were purchased from Sigma Chemical Co. (St Louis, MO). Phospholipase C suspended in 3.2 M-(NH_4) $_2\text{SO}_4$ was diluted to 100 U/ml in 150 mM-NaCl and stored at $0-5^\circ\text{C}$. Bovine LH (NIH-B9-LH) was supplied by NIH. Prostaglandin F-2 α (tromethamin salt) was obtained from Upjohn Co. (Kalamazoo, MI).

Statistics. Paired *t*-test and analysis of variance were used to determine the significance of treatment. The differences between groups were evaluated with Duncan's multiple range test.

Results

Flow cytometry and cell sorting

The use of flow cytometry made it possible to isolate fractions of highly purified large cell preparations. In Fig. 1 histograms of known purified small luteal cells obtained by unit gravity sedimentation (Koos & Hansel, 1981) are compared with the large cell enriched fraction. The

Fig. 1. Histograms of forward low-angle (FLA) light scattering, 90° scatter (90°SCT) and relative autofluorescence (RAF) of an impure large luteal cell fraction (a) compared with purified small luteal cells (b). The arrows indicate the cut-off points used to discriminate each cell type from the other.

Fig. 2. Two-parameter contour display of luteal cells using FLA at 488 nm (abscissa) versus log RAF (ordinate). The window setting used for sorting large luteal cells relative to the position of the other cell types is shown. The position of the cell types (small cells, cells <10 μ m and clumps) was obtained by sorting the cells from each of the areas indicated. Similar displays were observed in 8 independent experiments.

presence of large cells caused significant differences in forward low-angle scatter, 90° scatter, and relative autofluorescence, after excitation at 488 nm. A dual parameter mode, using forward low-angle scatter *versus* log relative autofluorescence was used to obtain the window (Fig. 2) for sorting the large luteal cells from other cells. Analysis of the total number of cells in these experiments indicated that the window containing pure preparations of large cells showed a high relative autofluorescence and consisted of only 2–6% of all the cells.

In unseparated preparations of mid-cycle (Day 10–12) luteal cells, the large luteal cells constituted only $5.1 \pm 0.8\%$ (mean $\pm$ s.e., $n = 8$) of all cells between 10 and 50 μ m in diameter. In the large cell-enriched fraction obtained by unit gravity sedimentation, large cells comprised only 25–35% ($28 \pm 4\%$, $n = 10$) of cells in the preparation. In contrast, the purity of large cells (Fig. 3) obtained by cell sorting was 90–99% ($92 \pm 1\%$, $n = 10$). Examination of the cells by electron microscopy revealed no morphological alterations due to sorting. Out of 10 CL, 2 failed to give a fluorescent signal sufficient to allow for a good separation. Sorting of cells from these two CL was difficult because the cells fluoresced weakly and the purity of the preparations obtained was 85%. These cells were not used in the incubations reported here. Only cell preparations with a purity >90% were used. The main contaminants of the preparations after sorting were occasional small cells attached to large luteal cells; this occurred in some preparations, while in others it was non-existent. Occasional clumps of small cells and endothelial cells occurred but these were <1% of the total cells. A typical sorting experiment resulted in 20–25 large luteal cells for every contaminating cell. The viability of the large cells was relatively low, ranging from 46 to 65% ($56 \pm 2\%$, $n = 10$). This was comparable to the viability of the large cell fraction before sorting, which was $63 \pm 3\%$ ($n = 8$).

Fig. 3. Photomicrograph of large luteal cells obtained by fluorescent activated cell sorting.

Effect of LH

Only very high doses of LH (100 ng/ml or more) increased ($P < 0.05$) progesterone production in the large cells. Progesterone production for 0, 5, 10, 100 and 1000 ng LH/ml was 1635 ± 318 , 2118 ± 699 , 2209 ± 256 , 2662 ± 459 and 3386 ± 335 pg/ 10^3 cells/4 h, respectively. In contrast, treatment of small cells with low doses of LH (1 and 5 ng/ml) stimulated ($P < 0.05$) progesterone production 6-fold (Table 1). Progesterone production was also stimulated 2-fold in large cells in the presence of 8-Br-cAMP (0.5 mM) and forskolin (1.0 μ M), as shown in Fig. 4.

Effects of PGF-2 α on progesterone production

When the small cells were incubated with various concentrations of PGF-2 α (0, 10, 100 and 1000 ng/ml), a dose-dependent increase in progesterone production resulted (Table 2). The progesterone stimulated by LH (1 ng/ml) was increased further ($P < 0.05$) when PGF-2 α was added.

In contrast, PGF-2 α , used at 1000 ng/ml, had no effect on basal progesterone production by the large cells (Fig. 4). However, progesterone production stimulated by LH, 8-Br-cAMP or forskolin was clearly inhibited ($P < 0.05$) by PGF-2 α .

Effects of phorbol ester, PBt₂

The phorbol ester, PBt₂, stimulated ($P < 0.05$) progesterone in small cells at a concentration of 50 nM; higher concentrations had no additional effect on steroidogenesis (Table 1). PBt₂ had no effect on LH-stimulated steroidogenesis. Similarly, PBt₂ plus the calcium ionophore A23187 resulted in progesterone production similar to PBt₂ alone (data not shown). As expected, the inactive phorbol ester, phorbol-13 monoacetate (PBAc), had no effect on basal or LH-stimulated progesterone synthesis.

Table 1. The effects of the active phorbol ester, PBT₂, or the inactive phorbol ester, phorbol-13 monoacetate (PBAc), with or without LH (1 or 5 ng/ml), on net progesterone production (ng/10⁵ cells/2 h) by small luteal cells

Phorbol ester (nM)	– LH	+ LH (1 ng/ml)	+ LH (5 ng/ml)
PBT ₂			
0	10.14 ± 2.97 ^a	58.47 ± 4.65 ^e	66.34 ± 5.72 ^e
1	13.95 ± 4.15 ^{a,b,c}	54.22 ± 4.64 ^e	—
10	11.98 ± 3.18 ^a	52.63 ± 4.03 ^e	—
20	13.53 ± 3.34 ^{a,b}	54.73 ± 2.02 ^e	59.70 ± 2.0 ^e
50	18.07 ± 5.07 ^{c,d}	51.91 ± 2.78 ^e	—
100	17.36 ± 4.24 ^{b,c,d}	52.14 ± 0.95 ^e	57.34 ± 0.66 ^e
200	18.83 ± 4.26 ^d	54.97 ± 2.01 ^e	—
500	19.66 ± 4.50 ^d	53.42 ± 1.30 ^e	—
PBAc			
100	10.2 ± 2.9 ^a	58.28 ± 1.13 ^e	—

Values are mean ± s.e.m. for 5 CL (–LH) or 3 CL (+LH) observations.
Values with the different superscripts differ (*P* < 0.05).

Fig. 4. Inhibition by PGF-2 α (1 μ g/ml) of the stimulatory effects of LH, 8-Br-cAMP and forskolin on progesterone production by large luteal cells. Values are means $\pm$ s.e. for 5 CL. **P* < 0.05, ***P* < 0.01, compared with progesterone production stimulated with LH, 8-Br-cAMP and forskolin in the absence of PGF-2 α .

In a related experiment (Table 3), the addition of exogenous phospholipase C (2 U/ml) also stimulated progesterone production in the small cells by nearly 2-fold compared with controls (*P* < 0.05). Surprisingly, in the presence of LH, this enzyme caused a significant (*P* < 0.05) inhibition of progesterone synthesis.

In contrast to results with small cells, addition of phorbol ester (20 or 100 nM) to large cells had no effect on progesterone production in the absence or presence of 5 ng LH/ml or 1 μ g LH/ml (Table 4).

Table 2. Effects of prostaglandin F-2 α on net production of progesterone (ng/10⁵ cells) by small luteal cells incubated for 2 h in the absence or presence of LH (1 ng/ml)

PGF-2 α (ng/ml)	– LH	+ LH (1 ng/ml)
0	19.88 $\pm$ 3.18 (6) ^a	82.51 $\pm$ 9.74 (4) ^d
10	28.48 $\pm$ 4.97 (6) ^a	88.75 $\pm$ 19.85 (4) ^d
100	36.90 $\pm$ 7.27 (6) ^b	95.79 $\pm$ 11.68 (4) ^{d,e}
1000	44.19 $\pm$ 9.28 (6) ^{b,c}	99.43 $\pm$ 7.73 (4) ^e

Values are mean $\pm$ s.e.m. for the no. of CL in parentheses.

Values within columns with different superscripts are significantly different ($P < 0.05$).

Table 3. The effects of phospholipase C (2 U/ml) on net progesterone production (ng/10⁵ cells/2 h) by small luteal cells

Treatment	– LH	+ LH (1 ng/ml)
Controls	18.90 $\pm$ 4.32 ^a	82.51 $\pm$ 9.74 ^c
Phospholipase C	32.79 $\pm$ 3.29 ^b	54.99 $\pm$ 5.21 ^d

Values are mean $\pm$ s.e.m. for 4 corpora lutea.

Values with different superscripts are significantly different ($P < 0.05$).

Table 4. The effects of phorbol ester, PBt₂, with or without LH, on net progesterone production (pg/10³ cells/4 h) by large luteal cells

PBt ₂ (nM)	– LH	LH (5 ng/ml)	LH (1 μ g/ml)
0	2231 $\pm$ 549	2915 $\pm$ 617	3447 $\pm$ 477
20	2224 $\pm$ 554	2601 $\pm$ 527	—
100	2403 $\pm$ 369	2700 $\pm$ 631	3413 $\pm$ 480

Values are mean $\pm$ s.e.m. for 5 corpora lutea.

Discussion

These experiments demonstrate the successful use of flow cytometry to obtain highly purified preparations of large bovine luteal cells by utilizing autofluorescence and forward light scatter as characteristic properties of these cells. It was impossible to separate cells without prior separation by unit gravity sedimentation. Our initial studies showed that the large luteal cells emit light

maximally at 578 nm when excited at 488 nm. We used forward low-angle light scatter at 488 nm, which is a function of cell size and shape, and the autofluorescence as dual parameters for cell sorting.

The purity of large cells obtained by this method is clearly superior to that of preparations produced by unit gravity sedimentation (Koos & Hansel, 1981) or elutriation (Fitz *et al.*, 1982; Harrison *et al.*, 1987). Unit gravity sedimentation yielded a large cell-enriched preparation which had 75% small cell contamination (Koos & Hansel, 1981), while elutriation yielded fractions of large cells that were $77 \pm 3\%$ pure (Harrison *et al.*, 1987). The yield of pure cells from flow cytometry used in this study was 90–99%.

The relatively low viability of the large cells was not caused by the cell sorter. The high levels of progesterone synthesis by the large cells during incubation indicates that most of these cells remained viable during incubation. Net progesterone production per cell was similar to that reported by Koos & Hansel (1981) for impure fractions of large cells.

The results, summarized in Table 5, clearly demonstrate a differential control of production of progesterone in large and small luteal cells in the cow. These results and those previously cited show that, apart from the well known cAMP second messenger system, the Ca^{2+} -PKC system (Nishizuka, 1984) is operational in the small luteal cells. The approximate doubling of progesterone biosynthesis seen previously with the total dispersed cell preparations (Hansel & Dowd, 1986) was observed in the present experiments with the small cells after the addition of the phorbol ester, PbT_2 , or phospholipase C. However, the calcium ionophore did not synergize with phorbol ester to increase progesterone production in the small cells, as suggested for other systems (Nishizuka, 1986). This suggests that in these cells, as reported for rat luteal cells (Baum & Rosberg, 1987), activation of protein kinase C stimulates progesterone production independently of elevation of calcium by ionophores.

Table 5. Summary of the effects of LH, PGF-2 α , a phorbol ester (PbT_2) and phospholipase C in the control of production of progesterone synthesis in small and large bovine luteal cells

Compound(s) added	Type of luteal cell	
	Small	Large
LH	+++	+
PGF-2 α	+	0
PbT_2	+	0
PGF-2 α + LH	++++	—
PbT_2 + LH	+++	0
Phospholipase C	+	ND

(+) = a 2-fold increase and (—) a 50% decrease in progesterone synthesis; 0 = no effect; ND = not determined.

The failure of an active phorbol ester to stimulate progesterone synthesis by the large luteal cells in these experiments, together with the fact that the stimulation found after adding the phorbol ester to small cells was equal to that found after its addition to total cell preparations, suggest that protein kinase C plays no essential role in large cells. However, further experiments including direct measurements of enzyme activity are needed to establish this point.

There were clear differences between the effects of PbT_2 and phospholipase C. PbT_2 increased progesterone synthesis when added at a level of 50 nM or higher, whereas phospholipase C, at the level used, was inhibitory to LH-stimulated progesterone production. Addition of phospholipase C increases polyphosphoinositide hydrolysis in luteal cells (Leung *et al.*, 1986). Other metabolic products, including prostanoids, are formed during this process via generation of diacylglycerol

(Majerus *et al.*, 1986). These prostanoids, including the products of the lipoxygenase pathway may exert an inhibitory action on steroidogenesis. For example, in an earlier study Milvae *et al.* (1986) showed that a product of the lipoxygenase pathway, 5-HETE, causes a reduction in basal and LH-stimulated progesterone synthesis in dispersed luteal cells. The predominant effect of PBT₂ is protein kinase C activation (Nishizuka, 1986). PBAC, however, has been shown (Takai *et al.*, 1984) not to activate protein kinase C, and it failed to stimulate progesterone production in small luteal cells in these experiments.

A major result of these experiments is the demonstration that the previously reported (Hixon & Hansel, 1979) stimulation of progesterone production by PGF-2 α in total dispersed cells is a property of the small, theca-derived cells. Benhim *et al.* (1987) also showed that PGF-2 α stimulated progesterone production in the small luteal cells. However, in contrast to our results, in which the LH-stimulated increase in small cells was further enhanced by PGF-2 α , progesterone production was inhibited in LH-stimulated cells in their studies which were carried out on CL of pregnant animals.

The maximal response achieved with PGF-2 α in small cells was similar to that achieved by addition of phorbol ester (PBT₂) or phospholipase C. PGF-2 α invokes its effect by a mechanism that appears to be independent of increases in cAMP (Speroff & Ramwell, 1970; Marsh, 1971; Davis *et al.*, 1987a). Davis *et al.* (1987b) demonstrated that PGF-2 α stimulates phosphatidylinositol 4,5-bisphosphate hydrolysis in the small cells, a process that also produces diacylglycerol, which activates protein kinase C (Nishizuka, 1986). Thus, the stimulatory effect of PGF-2 α on progesterone in bovine luteal cells appears to be mediated by a mechanism that involves activation of protein kinase C in the small luteal cells of theca cell origin (Alila & Hansel, 1984). However, it should be noted that PGF-2 α but not PBT₂, enhanced LH-stimulated progesterone production by the small cells. Reasons for these differences are unknown, but mechanisms of action other than protein kinase C activation may be involved.

Factors that regulate or maintain the high levels of progesterone production by the large cells are poorly understood. Our data showing that the large cells are responsive to stimulation by high levels (> 100 ng/ml) of LH, are qualitatively similar to those of Ursely & Leymarie (1979) and Koos & Hansel (1981) who used impure preparations of large cells. In contrast, small cells increased their progesterone output 6-fold in response to LH, and the maximum stimulatory dose was 5 ng/ml (Table 1).

Chegini *et al.* (1984) showed that basal, hCG- or cAMP-stimulated progesterone production and hCG binding were similar in the large and small luteal cells of CL of pregnancy. This finding is not surprising, considering that the majority of the large cells during pregnancy are derived from the small luteal cells of thecal origin (Alila & Hansel, 1984). Generally, cells from CL of pregnancy are less responsive to added LH than are those from CL collected during the oestrous cycle (Shemesh & Hansel, 1983).

Progesterone synthesis in large cells was stimulated 2-fold by forskolin and 8-Br-cAMP, suggesting that the cAMP system in these cells is indeed functional. Forskolin exerts its stimulatory effects mainly on the catalytic unit of adenylate cyclase (Seamon & Daly, 1981), although evidence in other tissues suggests that it may also stimulate the G/F protein regulation of the enzyme (Insel *et al.*, 1982). We do not know if all the large cells, or only some of them (the theca-derived cells, Alila & Hansel, 1984) are responsive. It is possible that the response to LH and other cAMP regulatory agents is due to the fact that, in the cow, the large luteal cells of theca cell derivation retain functional LH receptors.

Prostaglandin F-2 α alone had no effect on basal progesterone production by the large luteal cells; however, it inhibited LH, forskolin and 8-Br-cAMP-stimulated progesterone production. The ability of PGF-2 α to inhibit forskolin or 8-Br-cAMP-stimulated progesterone suggests that its effect occurs at some step distal to the intracellular generation of cAMP. Our observations on the effects of PGF-2 α on large cells are similar to those of Pate & Condon (1984) who showed that PGF-2 α inhibited progesterone synthesis in unseparated cultured bovine luteal cells. The inhibitory

effect of PGF-2 α on the large cells is in sharp contrast to its effect on small luteal cells, in which it stimulates progesterone synthesis, both alone and in combination with LH.

An implication of these findings on the function of the CL *in vivo* is that the large cells are the targets of the luteolytic action of PGF-2 α . The preponderance of the small cells, found in CL during early stages of the estrous cycle and their ability to respond to PGF-2 α by increasing progesterone production suggest that prostaglandins normally present in the bovine CL (Shemesh & Hansel, 1975; Milvae & Hansel, 1983) may play a paracrine role in the regulation of progesterone production.

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