Progesterone production, LH receptors, and oxytocin secretion by ovine luteal cell types on Days 6, 10 and 15 of the oestrous cycle and Day 25 of pregnancy*

L. M. Harrison†, N. Kenny and G. D. Niswender

Department of Physiology and Biophysics, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, Colorado 80523, U.S.A.

Summary. Corpora lutea were collected from sheep on Days 6, 10, and 15 of the oestrous cycle and Day 25 of pregnancy and dissociated into single cell suspensions. Purified preparations of large and small luteal cells were prepared by elutriation on all days except Day 6. Basal progesterone production by large cells was 6–8-fold higher than by small cells (36–65 vs 6–9 fg/cell/min). Oxytocin secretion was maximal on Day 6 (1.0 fg/cell/min) and declined thereafter. The number of receptors for LH increased between Day 6 and Day 10 and the two cell types had an equal number of receptors on Days 10 and 15 (19 000–23 000). Large cells on Day 25 of pregnancy had fewer receptors (12 000) than did small cells (26 000). Progesterone secretion by small luteal cells from all days examined was stimulated by LH (0.01–1000 ng/ml) in a dose-dependent manner; maximum sensitivity to LH occurred on Day 10. Despite the presence of receptors for LH on large cells, LH failed to stimulate progesterone production. Basal production of progesterone by large and small cells, and the response of small cells to LH, was not influenced by day examined. Re-combinations of large and small cells from Day 10 synergized to increase progesterone secretion. Prostaglandin E-2 (0.1–1000 ng/ml) did not stimulate progesterone secretion by large or small cells.

Introduction

The corpus luteum from a variety of mammalian species contains a number of cell types, two of which are steroidogenically competent, i.e. large luteal or granulosa-lutein cells, and small luteal or theca-lutein cells (Mossman & Duke, 1973). The two steroidogenic cell types have been characterized morphologically, separated on the basis of size, and shown to differ in their biochemical properties (Wilkinson *et al.*, 1976; Lemon & Loir, 1977; Ursely & Leymarie, 1979; Koos & Hansel, 1981; Fitz *et al.*, 1982; Rodgers & O'Shea, 1982; Chegini *et al.*, 1984; Rodgers *et al.*, 1984; Niswender *et al.*, 1985). Briefly, small luteal cells are 12–22 µm in diameter, stellate in shape, and characteristically contain an irregularly shaped nucleus, numerous mitochondria, lipid droplets, and abundant smooth endoplasmic reticulum (O'Shea *et al.*, 1979). In contrast, large luteal cells are spherical cells, 25–40 µm in diameter and are characterized by a greatly folded plasma membrane associated with a prominent basal lamina, a spherical nucleus, numerous mitochondria, lipid droplets, isolated stacks of rough endoplasmic reticulum and an abundance of the smooth endoplasmic reticulum characteristic of steroidogenic cells. Large cells uniquely contain electron-dense secretory granules, which, based on immunocytochemical data, appear to be the source of luteal oxytocin (Guldenaar

*Reprint requests to Dr G. D. Niswender.

†Present address: Department of Paediatrics, C232 University of Colorado Health Sciences Center, 4200 East 9th Avenue, Denver, Colorado 80262, U.S.A.

et al., 1984; Rice & Thorburn, 1985; Sawyer *et al.*, 1986; Theodosis *et al.*, 1986). The majority of receptors for LH have been reported to be on small luteal cells, whereas the majority of receptors for oestradiol and prostaglandins (PG) E-2 and F-2 α are located on large luteal cells (Fitz *et al.*, 1982; Glass *et al.*, 1984). Although unresponsive to LH, the basal rate of secretion of progesterone (i.e. unstimulated) from large luteal cells is greater than that from small cells (Fitz *et al.*, 1982; Schwall *et al.*, 1986a). In contrast, small luteal cells are highly responsive to the stimulatory action of LH (Fitz *et al.*, 1982; Rodgers *et al.*, 1983a). Both cell types possess a stimutable adenylate cyclase system, but in the large cell stimulation of this enzyme does not increase synthesis or secretion of progesterone (Hoyer *et al.*, 1984; Hoyer & Niswender, 1985).

Much of the information concerning large and small luteal cell function has been obtained using corpora lutea removed at mid-cycle (Days 8–12 of the oestrous cycle), or tissue collected from slaughter houses, for which the precise age of the corpora lutea was not known. The corpus luteum is morphologically and biochemically dynamic over the course of the oestrous cycle, exhibiting fluctuation in the number of receptors for LH (Diekman *et al.*, 1978), secretion of progesterone (Hansel *et al.*, 1973), numbers of large and small luteal cells (Farin *et al.*, 1986; Schwall *et al.*, 1986b) and responsiveness to PGF-2 α (Hearnshaw *et al.*, 1973). It is possible that large and small cell responses to trophic/lytic stimuli change over the course of the oestrous cycle and into pregnancy.

The objectives for the present experiment were to use purified large and small luteal cells from defined stages of the oestrous cycle and early pregnancy to: (a) quantitate the number of receptors for LH; (b) measure in-vitro secretion of oxytocin and production of progesterone in response to LH and PGE-2; and (c) determine whether interactions occur between large and small luteal cells which affect the production of oxytocin or progesterone.

Materials and Methods

Materials. Type IV collagenase was purchased from Cooper Biomedical (Malvern, PA). Medium 199 was from Grand Island Biochemical Co. (Grand Island, NY). Prostaglandin E-2 was obtained from the Upjohn Company (Kalamazoo, MI). The Na¹²⁵I used for radioiodinations was purchased from Amersham (Arlington Heights, IL). Oxytocin used for the standards and ¹²⁵I-labelled tracer in radioimmunoassays, and BSA (fraction V) were purchased from Sigma Chemical Co. (St Louis, MO).

Tissue preparation. Suspensions of purified large and small luteal cells were prepared by a modification of previously described methods (Fitz *et al.*, 1982; Schwall *et al.*, 1986b). Briefly, corpora lutea were surgically removed from Western range ewes on Days 6, 10 and 15 of the oestrous cycle and Day 25 of pregnancy, sectioned into 0.5 mm slices using a Stadie-Riggs hand microtome, and the tissue pooled by day of oestrous cycle or pregnancy (4–6 corpora lutea per tissue pool). Sliced CL were incubated in Medium 199 containing 0.2% collagenase (145 U/mg) and 0.05% deoxyribonuclease, and gently agitated for 2–3 h at 37°C in a water bath to yield a suspension of single cells. The dissociated cells were washed in Medium 199 (containing 20 mM-Hepes, 0.1% BSA, 0.35 mg NaHCO₃/ml, 100 U penicillin/ml, and 0.2 mg streptomycin sulphate/ml, pH 7.35) before large and small cell fractions were separated by centrifugal elutriation. Corpora lutea removed on Day 6 of the oestrous cycle were dissociated but the cells were not subjected to elutriation since morphologically distinct large and small cells do not differ sufficiently in size to be separated by this technique (M. H. Mayan & G. D. Niswender, unpublished observations).

Experimental procedures. After dissociation and elutriation aliquants were removed from each pool of purified cells and (a) the numbers of large and small cells were counted after placement on a haemocytometer, (b) viability was assessed using acridine orange stain, and (c) the number of steroidogenic cells in each fraction was measured as indicated by positive staining for 3 β -hydroxysteroid dehydrogenase (3 β -HSD) activity (Payne *et al.*, 1980). Triplicate aliquants of 10 000 large cells, 50 000 small cells or 10 000 large cells plus 50 000 small cells were removed from each purified cell fraction and dispensed into 12 $\times$ 75 mm glass tubes containing 0, 0.1, 1, 10, 100 or 1000 ng PGE-2/ml or 0.01, 0.1, 1, 10, 100 or 1000 ng LH/ml in Medium 199 in a total volume of 1 ml. Cells were incubated for 2 h at 37°C in a shaking water bath, and incubations were stopped by freezing. Samples were stored at –20°C until assayed for progesterone and oxytocin. Purified cells not used for in-vitro incubations were frozen in Medium 199 at –70°C until receptors for LH were quantified.

Replication. The experiment detailed above was replicated 4 times between 2nd December 1985 and 9th January 1986 and used a total of 96 corpora lutea from 68 spontaneously ovulating ewes.

Radioimmunoassays. Cell incubations were thawed at room temperature and vortexed before the cellular compartment was pelleted by centrifugation. Progesterone was measured in unextracted aliquants of media by radioimmunoassay (Niswender, 1973). The sensitivity of the progesterone assay was 40 pg/tube and the intra- and inter-assay coefficients of variation were 11% and 18% respectively. Oxytocin in unextracted aliquants of medium was measured using the procedures and antiserum of Robinson (1980). The sensitivity of the oxytocin assay was 4 pg/tube and the intra- and inter-assay coefficients of variation were 16% and 22%, respectively.

Receptor assays. Receptors for LH were quantified using the standard curve technique (Braden *et al.*, 1986). Briefly, a standard luteal membrane pool was prepared by homogenizing corpora lutea from superovulated ewes in sucrose buffer using a Polytron and a Dounce homogenizer and the membrane fraction was recovered by centrifugation. The number of receptors in the standard membrane preparation was determined by Scatchard analysis (Scatchard, 1949) of data obtained by incubating various concentrations of ^{125}I -labelled hCG with 5 mg of the standard membrane preparation. Non-specific binding was determined by adding a 200-fold excess of hCG to incubation tubes containing tissue and ^{125}I -labelled hCG. To quantitate receptor number in experimental tissue, samples of purified luteal cells (1×10^5 small luteal cells or 2×10^5 large luteal cells) were homogenized using a Dounce homogenizer and triplicate samples were incubated with 7 fmol ^{125}I -labelled hCG. The ^{125}I -labelled hCG specifically bound was compared with a standard curve generated by incubating the same amount of ^{125}I -labelled hCG with increasing quantities (1–50 mg) of the standard membrane preparation. The membranes were incubated for 16 h, 4 ml cold assay buffer were added to each tube, and free ^{125}I -labelled hCG was separated from that bound to the membrane pellet by centrifugation (30 min at 30 000 g). The supernatant was then decanted and the radioactivity in the pellet counted. The number of receptors was determined by comparing the counts specifically bound in the experimental tissue with the counts specifically bound per mg of standard membrane preparation, which had a known concentration of receptors.

Analysis of data. Data were subjected to analysis of variance using the General Linear Model procedure of the Statistical Analysis System (Cary, NC) and least squares means obtained for comparison of treatments across replicates.

Results

Separation of cell types

Purification by elutriation of small luteal cells yielded fractions that were $97 \pm 0.7\%$ small cells, of which $96 \pm 0.9\%$ stained positive for $3\beta\text{-HSD}$ activity. Similarly, the large cell fractions were composed of $77 \pm 3\%$ large luteal cells of which $90 \pm 3\%$ were positive for $3\beta\text{-HSD}$ (Table 1). The yield of cells per gram of luteal tissue subjected to dissociation and purification is shown in Table 2. Viability of dissociated or elutriated luteal cells (as determined by staining with acridine orange) was always greater than 85% for both large and small cell fractions regardless of the age of the corpora lutea. Additionally, none of the hormonal treatments, or incubation conditions, adversely affected cell viability over the time course of these experiments.

Table 1. Percentage of luteal cell types in elutriated cell fractions from sheep at Days 10 and 15 of the oestrous cycle and Day 25 of pregnancy*

	Day 10		Day 15		Day 25	
	Large	Small	Large	Small	Large	Small
Large cells	69 (83)	31 (17)	84 (95)	16 (5)	79 (92)	21 (8)
Small cells	4 (5)	96 (95)	2 (3)	98 (97)	3 (3)	97 (97)

Values are % of large and small luteal cells in each fraction (endothelial cells, fibroblasts etc. are not considered), and are mean values from 4 replications; s.e.m. values were all <6%.

Values in parentheses represent % of cells in that fraction that stained for $3\beta\text{-HSD}$ activity.

*Cell suspensions obtained on Day 6 of the cycle were 31% $3\beta\text{-HSD}$ -positive large luteal cells and 69% $3\beta\text{-HSD}$ -positive small luteal cells.

Table 2. Yields of large and small cells after dissociation and elutriation of corpora lutea from Days 6, 10 and 15 of the oestrous cycle and Day 25 of pregnancy

Day	Cell	10 ⁶ cells/g CL	Large:small cell ratio
6*	Small	25.5 ± 2.8	1:3.7
	Large	6.8 ± 0.4	
10	Small	9.7 ± 1.3	1:3.0
	Large	3.2 ± 0.3	
15	Small	6.0 ± 1.3	1:2.0
	Large	3.0 ± 0.3	
25	Small	6.3 ± 0.7	1:2.7
	Large	2.3 ± 0.2	

Values are mean ± s.e.m. for 4 replicates.

*Day 6 CL were only dissociated.

Table 3. Progesterone production by small luteal cells from different stages of the oestrous cycle (Days 6, 10 and 15) and early pregnancy (Day 25) after treatment with LH

LH (ng/ml)	Progesterone production (fg/cell/min)*			
	Day 6†	Day 10	Day 15	Day 25
0.0	32.6 ^a _d	6.7 ^b _d	9.2 ^b _d	5.7 ^b _d
0.01	30.3 ^a _d	8.8 ^b _d	5.7 ^b _d	6.0 ^b _d
0.1	41.3 ^a _d	23.3 ^b _d	9.8 ^c _d	9.8 ^c _d
1.0	47.5 ^a _e	41.5 ^b _f	25.0 ^c _e	24.5 ^c _e
10.0	53.2 ^a _e	43.0 ^b _f	32.5 ^c _e	32.0 ^c _{eg}
100.0	52.5 ^a _e	42.0 ^b _f	35.3 ^b _{fg}	34.7 ^b _{fg}
1000.0	49.2 ^a _e	40.3 ^b _f	28.9 ^b _{eg}	29.2 ^b _{eg}

*Pooled standard error of the mean = 3.6, *n* = 4.

†Day 6 corpora lutea were dissociated but not elutriated; the mixed cell population comprised large and small cells in a 1:3.7 ratio (see Table 2) and the high basal production of progesterone in this group is assumed to be of large cell origin.

Values in the same row with different superscripts (a,b,c) differ (*P* < 0.05).

Values in the same column with different subscripts (d,e,f,g) differ (*P* < 0.05).

Progesterone production

The basal rate of production of progesterone (Tables 3–6) from purified large cells was greater than from small cells on Day 10 and 15 of the cycle and Day 25 of pregnancy (*P* < 0.01). The amount of progesterone produced by each cell type did not change for any of these days of the cycle or pregnancy (*P* > 0.10). When LH was included in the incubation medium, both day of cycle and dose of LH affected the amount of progesterone produced by small luteal cells (*P* < 0.01; Table 3). Production of progesterone by small cells from Day 10 of the oestrous cycle was enhanced at a lower dose of LH than in small cells from Day 15 or Day 25 of pregnancy (0.1 vs 1.0 ng/ml; *P* < 0.01). Small cells from Day 10 produced more progesterone in response to 1 and 10 ng LH/ml

Fig. 1. Production of progesterone by Day 10 large and small luteal cells incubated separately or together. *Significantly elevated compared to sum of separate incubations; $P < 0.01$. Pooled s.e.m. = 22.7, $n = 4$.

Table 4. Progesterone production by large luteal cells from different stages of the oestrous cycle (Days 10 and 15) and early pregnancy (Day 25) after treatment with LH

LH (ng/ml)	Progesterone production (fg/cell/min)*		
	Day 10	Day 15	Day 25
0.0	50.0 ^a	66.7 ^a	35.8 ^a
0.01	59.2 ^a	65.8 ^a	22.5 ^b
0.1	47.5 ^a	64.2 ^a	20.0 ^b
1.0	61.7 ^a	59.2 ^a	23.3 ^b
10.0	51.7 ^a	68.3 ^a	25.8 ^b
100.0	64.2 ^{ab}	74.2 ^a	28.3 ^b
1000.0	51.7 ^{ab}	84.2 ^a	35.8 ^b

*Pooled standard error of the mean = 8.9, $n = 4$.

Values in the same row with different superscripts (a,b) differ ($P < 0.05$).

than did small cells from Day 15 of the oestrous cycle or Day 25 of pregnancy ($P < 0.05$). Maximal stimulation of progesterone production was obtained at 10 ng LH/ml for Day 10 small cells and at 100 ng LH/ml for Day 15 and Day 25 pregnant small cells, but the absolute amount of progesterone produced in response to LH did not differ between days ($P > 0.10$). Generally, when the purified large and small cells were incubated together the amount of progesterone produced in response to LH did not differ significantly from the sum of the progesterone produced by large cells and small cells when incubated separately. However, mixed cells from Day 10 produced significantly greater amounts of progesterone at the higher LH doses (10, 100 and 1000 ng/ml) than would be predicted from the incubation of each individual cell type ($P < 0.01$; Fig. 1).

In contrast, large luteal cells did not respond to LH with an increase in progesterone production, regardless of the dose of LH used or the stage of the cycle or pregnancy (Table 4).

Inclusion of PGE-2 in luteal cell cultures did not increase progesterone production by large or small luteal cells, and actually inhibited production at higher doses (Tables 5 & 6).

Table 5. Progesterone production by large luteal cells from different stages of the oestrous cycle (Days 10 and 15) and early pregnancy (Day 25) after treatment with PGE-2

PGE-2 (ng/ml)	Progesterone production (fg/cell/min)*		
	Day 10	Day 15	Day 25
0.0	50.0 ^a _c	66.7 ^a _c	35.8 ^a _c
0.1	47.5 ^a _c	61.7 ^a _c	24.2 ^b _c
1.0	52.5 ^a _c	56.7 ^a _c	21.7 ^b _c
10.0	53.3 ^a _c	50.8 ^a _c	23.3 ^b _c
100.0	38.3 ^a _b _c	47.5 ^a _c	23.3 ^b _c
1000.0	40.0 ^a _c	26.7 ^a _d	20.0 ^a _c

*Pooled standard error of the mean = 8.8, $n = 4$.

Values in the same row with different superscripts (a,b) differ ($P < 0.05$).

Values in the same column with different subscripts (c,d) differ ($P < 0.05$).

Table 6. Progesterone production by small luteal cells from different stages of the oestrous cycle (Days 6, 10 and 15) and early pregnancy (Day 25) after treatment with PGE-2

PGE-2 (ng/ml)	Progesterone production (fg/cell/min)*			
	Day 6†	Day 10	Day 15	Day 25
0.0	32.6 ^a _c	6.7 ^b _c	9.2 ^b _{cd}	5.7 ^b _c
0.1	29.3 ^a _c	7.7 ^b _c	10.2 ^b _c	6.3 ^b _c
1.0	30.7 ^a _c	8.2 ^b _c	6.3 ^b _d	9.2 ^b _d
10.0	31.1 ^a _c	6.2 ^b _c	5.3 ^b _d	5.2 ^b _c
100.0	28.1 ^a _c	8.0 ^b _c	5.5 ^b _d	5.7 ^b _c
1000.0	21.8 ^a _d	4.8 ^b _c	5.2 ^b _d	3.2 ^b _c

*Pooled standard error of the mean = 1.1, $n = 4$.

†Day 6 corpora lutea were dissociated but not elutriated; the mixed cell population comprised large and small cells in a 1:3.7 ratio (see Table 2) and the high basal production of progesterone in this group is assumed to be of large cell origin.

Values in the same row with different superscripts (a,b) differ ($P < 0.05$).

Values in the same column with different subscripts (c,d) differ ($P < 0.05$).

Receptors for LH

On a per cell basis the two luteal cell populations did not differ in their ability to specifically bind hCG on Days 10 and 15 (Table 7). The large luteal cells from these days had as many receptors for LH/hCG as did small luteal cells. Mixed cells from Day 6 (predominantly small cells) had fewer receptors than did either large or small cells from other days of the cycle ($P < 0.05$). There were more receptors for LH on small luteal cells than large luteal cells prepared on Day 25 of pregnancy.

Oxytocin production

Oxytocin was detectable only in the cell suspensions which contained large cells (or mixed cells). Production of oxytocin was maximal in cell suspensions from Day 6, declined significantly by Day

Table 7. Distribution of receptors for LH on large and small luteal cell types on Days 6, 10 and 15 of the oestrous cycle and Day 25 of pregnancy

Day	No. of receptors/cell*	
	Large cells	Small cells
6	12 784 ± 2865†	
10	22 782 ± 1522 ^a	19 062 ± 2121 ^a
15	21 215 ± 6904 ^a	21 396 ± 6898 ^a
25	12 477 ± 1246 ^b	25 659 ± 3651 ^a

*Mean ± s.e.m., *n* = 4.

†Cells not elutriated (see text).

Means with different superscripts differ, *P* < 0.05.**Table 8.** Oxytocin production by luteal cells of sheep on Days 6, 10 and 15 of the oestrous cycle and Day 25 of pregnancy

Day	Oxytocin (ng/ml)*†		
	Mixed cells‡	Large cells	Small cells
6	1.31 ^a	(cells were not elutriated)	
10	0.55 ^b	0.59 ^b	<0.004 ^d
15	0.11 ^c	0.07 ^c	<0.004 ^d
25	<0.004 ^d	<0.004 ^d	<0.004 ^d

*Pooled standard error of the mean = 0.09, *n* = 4.

†Oxytocin secretion was not affected by treatment with LH or PGE-2; values are pooled within cell type and day of cycle.

‡Mixed cells from Day 6 corpora lutea were dissociated, but not elutriated; mixed cells from Days 10, 15 and 25 were purified large (10 000) and small (50 000) luteal cells added back together.

Means with different superscripts (a,b,c,d) differ (*P* < 0.05).

10, and was very low or undetectable in large cell or mixed cell cultures on Day 15 of the oestrous cycle and Day 25 of pregnancy. Neither LH nor PGE-2 affected oxytocin production regardless of the dose used or the day of the cycle; therefore the oxytocin values were pooled by day and cell type (Table 8).

Discussion

The purity of the large and small luteal cell fractions illustrates the efficacy of centrifugal elutriation for separating cells of different size/density (Table 1). The degree of purity of both cell types was improved compared to that reported by Fitz *et al.* (1982) using elutriation, or for that achieved using Ficoll gradients (Rodgers & O'Shea, 1982). The yield of cells per gram of ovarian tissue subjected to dissociation and purification was comparable to that in these two previous reports (Table 2).

LH receptors were measured by the standard curve technique; this method allows accurate estimation of receptor number in small amounts of tissue (inadequate for Scatchard analysis), and we have found a correlation of >0.97 between estimation of receptor numbers by this method and

by Scatchard analysis for LH receptors (Braden *et al.*, 1986). The presence of equal numbers of receptors for LH on large and small luteal cells on Days 10 and 15 of the oestrous cycle is not in agreement with previous data from this laboratory (Fitz *et al.*, 1982) reporting that receptors for LH were located primarily on small cells. This apparent discrepancy may be due to the use of luteal cells from spontaneously ovulating ewes in the present experiment compared to the corpora lutea from animals induced to superovulate with PMSG used by Fitz *et al.* (1982). Administration of PMSG to ewes increases the number of luteinized follicles, alters the cells, and may induce loss of luteinizing granulosa cells, leading to corpora lutea composed of hypertrophied theca-lutein cells (Cran, 1983). In fact, Fitz *et al.* (1982) reported that the number of receptors for LH was similar on large and small cells from anoestrous ewes treated with PMSG and hCG.

Donaldson & Hansel (1965) first suggested that small luteal cells differentiate into large luteal cells, and it has been demonstrated that LH may induce the differentiation of small cells into large cells (Farin *et al.*, 1985). Injection of LH into ewes during the mid-luteal phase of the cycle increased the number of large cells and concomitantly reduced the number of small cells in the ovine corpus luteum. Given the abundance of receptors for LH on small cells, and that large cells may arise from small cells *in vivo*, the presence of receptors for LH on large cells may not be surprising. Additionally, procedural differences may have affected receptor binding or number of receptors. Complete Hanks' medium was used for dissociation and elutriation in the present experiment whereas Fitz *et al.* (1982) used calcium and magnesium-free Hanks' medium. O'Shaughnessy & Wathes (1985), using bovine luteal cells in culture, showed that medium constituents have a pronounced effect on cell function and response.

The present study provides evidence that, despite the presence of receptors for LH on large cells, LH fails to stimulate large cells to secrete either progesterone or oxytocin. Likewise, as previously reported (Hoyer *et al.*, 1984), neither cAMP nor adenylate cyclase activity is increased in large cells treated with LH. At the present time the function of the receptors for LH on large luteal cells is unknown.

The increase in the number of receptors for LH from Days 6 to 10 without subsequent changes on Day 15 of the oestrous cycle or Day 25 of pregnancy suggests that, by mid-cycle, luteal cells have attained their maximum complement of receptors for LH. This is consistent with the data of Diekman *et al.* (1978). The increased sensitivity of Day 10 small cells to LH (compared with Day 15 of the oestrous cycle or Day 25 of pregnancy) supports the concept that events within the cell that occur subsequent to hormone binding change during the oestrous cycle and pregnancy, and may be important in modulating the magnitude of the response to LH. It is significant that the greater sensitivity of the Day 10 small cells to LH *in vitro* corresponds with the time when progesterone secretion is greatest *in vivo* (Hansel *et al.*, 1973; Diekman *et al.*, 1978).

The relevance of the synergism in progesterone production between large and small cells collected on Day 10 is not clear. Interaction between the two steroidogenic luteal cell types has previously been reported for the sow (Lemon & Mauléon, 1982), but the factor(s) responsible for this interaction has not been identified. Such interaction between the two luteal cell types may be analogous to the interaction between the granulosa and theca cells that apparently plays a role in regulating progesterone synthesis in the hamster follicle (Makris & Ryan, 1977). Granulosa and theca cells co-operate in follicular oestrogen production (see review by Hillier, 1981). Large and small cells are probably derived from granulosa and theca cells respectively (O'Shea *et al.*, 1980). It is therefore possible that luteal progesterone production is achieved by an analogous interaction between large and small luteal cells. Although the results of Rodgers *et al.* (1985) provided no evidence for large and small ovine luteal cell interaction in progesterone synthesis, their studies utilized plated cells from corpora lutea of undetermined stages of the oestrous cycle. It seems likely, therefore, that the interaction of large and small luteal cells warrants further investigation to elucidate any possible interactive or co-operative roles of these two cell types.

In contrast to previous studies in this laboratory treatment with PGE-2 did not stimulate progesterone production from large luteal cells at any dose or day of the cycle or pregnancy examined.

Fitz *et al.* (1984a, b) and Silvia *et al.* (1984) found that large cells can be stimulated to produce progesterone by PGE-2, but these studies used plated luteal cells which were exposed to PGE-2 for 3 h or more. It is possible that culture conditions or duration of exposure to PGE-2 affect the actions of this prostaglandin on luteal cells.

Our observation that only large cells produce oxytocin confirms previous reports (Rodgers *et al.*, 1983b; Sawyer *et al.*, 1986) and demonstrates that maximal secretion of oxytocin occurs early in the oestrous cycle. Ivell *et al.* (1985) reported high quantities of mRNA for oxytocin in the bovine post-ovulatory follicle which declined as the corpus luteum developed; however, maximal quantities of ovarian oxytocin do not appear in the circulation earlier than 4–5 days after ovulation (Schams *et al.*, 1982). These observations are in accordance with the recent data of Jungclas & Luck (1986) that oxytocin production is maximal in 3-day-old cultures of granulosa cells harvested from ovulatory cow follicles, and with the present results indicating that Day 6 sheep luteal cells produce significant amounts of oxytocin. Wathes *et al.* (1983) extracted and measured the oxytocin present in bovine corpora lutea obtained at slaughter; those corpora lutea judged to be 5–10 days old contained more oxytocin than did corpora lutea from any other stage of the cycle. These studies provide strong evidence that maximum production of oxytocin in ewes and cows occurs early in the oestrous cycle. Although luteal oxytocin may play a role in modulating secretion of PGF-2 α during luteal regression as suggested by Flint & Sheldrick (1983), the physiological relevance of high concentrations of oxytocin earlier in the oestrous cycle remains undetermined.

We thank Dr M. H. Mayan and Dr F. Gamboni for help with the tissue preparation and the receptor assays; Kathy Miller for preparation of the figure; the National Institute of Health (Bethesda, MD) for the ovine LH (S-24); and Dr I. A. Robinson (National Institute of Medical Research, Mill Hill, London, England) for the anti-oxytocin serum. This work was supported in part by NIH grant HD 11590, by NIH Training Grant HD 07031 (to L.M.H.), and SERC/NATO Fellowship B/RF/6952 (to N.K.).

References

- Braden, T.D., Manns, J.G., Cermak, D.L., Nett, T.M. & Niswender, G.D. (1986) Follicular development following parturition and during the oestrous cycle in beef cows. *Theriogenology* **25**, 833–843.
- Cegini, N., Ramani, N. & Rao, Ch.V. (1984) Morphological and biochemical characterization of small and large bovine luteal cells during pregnancy. *Molec. cell. Endocr.* **37**, 89–102.
- Cran, D.G. (1983) Follicular development in the sheep after priming with PMSG. *J. Reprod. Fert.* **67**, 415–423.
- Diekman, M.A., O'Callaghan, P., Nett, T.M. & Niswender, G.D. (1978) Validation of methods and quantification of luteal receptors for LH throughout the oestrous cycle and early pregnancy in ewes. *Biol. Reprod.* **19**, 999–1009.
- Donaldson, L. & Hansel, W. (1965) Histological study of bovine corpora lutea. *J. Dairy Sci.* **48**, 905–909.
- Farin, C.E., Schwall, R.H., Gamboni, F., Sawyer, H.R. & Niswender, G.D. (1985) Effect of LH and hCG on size distributions of luteal cells in the cycling ewe. *Biol. Reprod.* **32** (Suppl. 1), 13, Abstr.
- Farin, C.E., Moeller, C.L., Sawyer, H.R., Gamboni, F. & Niswender, G.D. (1986) Morphometric analysis of cell types in the ovine corpus luteum throughout the oestrous cycle. *Biol. Reprod.* (in press).
- Fitz, T.A., Mayan, M.H., Sawyer, H.R. & Niswender, G.D. (1982) Characterization of two steroidogenic cell types in the ovine corpus luteum. *Biol. Reprod.* **27**, 703–711.
- Fitz, T.A., Hoyer, P.B. & Niswender, G.D. (1984a) Interactions of prostaglandins with sub-populations of ovine luteal cells. I: Stimulatory effects of prostaglandins E-1, E-2, and I-2. *Prostaglandins* **28**, 119–126.
- Fitz, T.A., Mock, E.J., Mayan, M.H. & Niswender, G.D. (1984b) Interactions of prostaglandins with sub-populations of ovine luteal cells. II: Inhibitory effects of PGF-2 α and protection by PGE-2. *Prostaglandins* **28**, 127–138.
- Flint, A.P.F. & Sheldrick, E.L. (1983) Evidence for a systemic role for ovarian oxytocin in luteal regression in sheep. *J. Reprod. Fert.* **67**, 215–225.
- Glass, J.D., Fitz, T.A. & Niswender, G.D. (1984) Cytosolic receptor for estradiol in the corpus luteum of the ewe: variation throughout the oestrous cycle and distribution between large and small steroidogenic cell types. *Biol. Reprod.* **31**, 967–974.
- Guldenaar, S.E.F., Wathes, D.C. & Pickering, B.T. (1984) Immunocytochemical evidence for the presence of oxytocin and neurophysin in the large cells of the bovine corpus luteum. *Cell Tissue Res.* **237**, 349–352.

- Hansel, W., Concannon, P.W. & Lukaszewska, J.H. (1973) Corpora lutea of the large domestic animals. *Biol. Reprod.* **8**, 222–245.
- Hearnshaw, H., Restall, B.J. & Gleeson, A.R. (1973) Observations on the luteolytic effects of prostaglandin F-2 α during the oestrous cycle and early pregnancy in the ewe. *J. Reprod. Fert.* **32**, 322–323.
- Hillier, S.G. (1981) Regulation of follicular oestrogen synthesis; a survey of current concepts. *J. Endocr.* **89**, 3P–18P.
- Hoyer, P.B. & Niswender, G.D. (1985) The regulation of steroidogenesis is different in the two types of ovine luteal cells. *Can. J. Physiol. Pharmacol.* **63**, 240–248.
- Hoyer, P.B., Fitz, T.A. & Niswender, G.D. (1984) Hormone independent activation of adenylate cyclase in large steroidogenic ovine luteal cells does not result in increased progesterone synthesis. *Endocrinology* **114**, 604–608.
- Ivell, R., Brackett, K.H., Fields, M.J. & Richter, D. (1985) Ovulation triggers oxytocin expression in the bovine ovary. *FEBS Letters* **190**, 263–267.
- Jungclas, B. & Luck, M.R. (1986) Evidence for granulosa-theca interaction in the secretion of oxytocin by bovine ovarian tissue. *J. Endocr.* **109**, R1–R4.
- Koos, R.D. & Hansel, W. (1981) The large and small cells of the bovine corpus luteum: ultrastructural and functional differences. In *Dynamics of Ovarian Function*, pp. 197–203. Eds N. B. Schwartz & M. Hunzicker-Dunn. Raven Press, New York.
- Lemon, M. & Loir, M. (1977) Steroid release *in vitro* by two luteal cell types in the corpus luteum of the pregnant sow. *J. Endocr.* **72**, 351–359.
- Lemon, M. & Mauléon, P. (1982) Interaction between two luteal cell types from the corpus luteum of the sow in progesterone synthesis *in vitro*. *J. Reprod. Fert.* **64**, 315–323.
- Makris, A. & Ryan, K.J. (1977) Evidence for interaction between granulosa and theca cells in early progesterone synthesis. *Endocr. Res. Commun.* **4**, 233–246.
- Mossman, H.W. & Duke, K.L. (1973) Some comparative aspects of the mammalian ovary. In *Handbook of Physiology*, Section 7: *Endocrinology*, Vol. 2, Part 1, pp. 389–402. Ed S. R. Geiger. American Physiological Society, Washington, DC.
- Niswender, G.D. (1973) Influence of the site of conjugation on the specificity of antibodies to progesterone. *Steroids* **22**, 413–424.
- Niswender, G.D., Schwall, R.H., Fitz, T.A., Farin, C.E. & Sawyer, H.R. (1985) Regulation of ovarian function in domestic ruminants: New concepts. *Recent Prog. Horm. Res.* **41**, 101–151.
- O'Shaughnessy, P.J. & Wathes, D.C. (1985) Characteristics of bovine luteal cells in culture: morphology, proliferation, and progesterone secretion in different media and effects of LH, dibutyryl cyclic AMP, antioxidants and insulin. *J. Endocr.* **104**, 355–361.
- O'Shea, J.D., Cran, D.G. & Hay, M.F. (1979) The small luteal cell of the sheep. *J. Anat.* **128**, 239–251.
- O'Shea, J.D., Cran, D.G. & Hay, M.F. (1980) Fate of the theca interna following ovulation in the ewe. *Cell Tissue Res.* **210**, 305–319.
- Payne, A.H., Downing, J.R. & Wong, K.-L. (1980) Luteinizing hormone receptors and testosterone synthesis in two distinct populations of Leydig cells. *Endocrinology* **106**, 1424–1430.
- Rice, G.E. & Thorburn, G.D. (1985) Subcellular localization of oxytocin in the ovine corpus luteum. *Can. J. Pharmacol.* **63**, 309–314.
- Robinson, I.C.A.F. (1980) The development of a sensitive and specific radioimmunoassay for oxytocin in unextracted plasma. *J. Immunoassay* **1**, 323–347.
- Rodgers, R.J. & O'Shea, J.D. (1982) Purification, morphology, and progesterone production and content of three cell types isolated from the corpus luteum of the sheep. *Aust. J. Biol. Sci.* **35**, 441–445.
- Rodgers, R.J., O'Shea, J.D. & Findlay, J.K. (1983a) Progesterone production *in vitro* by small and large ovine luteal cells. *J. Reprod. Fert.* **69**, 113–124.
- Rodgers, R.J., O'Shea, J.D., Findlay, J.K., Flint, A.P.F. & Sheldrick, E.L. (1983b) Large luteal cells the source of luteal oxytocin in the sheep. *Endocrinology* **113**, 2302–2304.
- Rodgers, R.J., O'Shea, J.D. & Bruce N.W. (1984) Morphometric analysis of the cellular composition of the ovine corpus luteum. *J. Anat.* **138**, 757–769.
- Rodgers, R.J., O'Shea, J.D. & Findlay, J.K. (1985) Do small and large luteal cells of the sheep interact in the production of progesterone? *J. Reprod. Fert.* **75**, 85–94.
- Sawyer, H.R., Moeller, C.L. & Kozlowski, G.P. (1986) Immunocytochemical localization of neurophysin and oxytocin in ovine corpora lutea. *Biol. Reprod.* **34**, 543–548.
- Scatchard, G. (1949) The attraction of proteins for small molecules and ions. *Ann. N.Y. Acad. Sci.* **51**, 660–672.
- Schams, D., Lahlou-Kassi, A. & Glatzel, P. (1982) Oxytocin concentrations in the peripheral blood during the oestrous cycle and after ovariectomy in two breeds of sheep with low and high fecundity. *J. Endocr.* **92**, 9–13.
- Schwall, R.H., Sawyer, H.R. & Niswender, G.D. (1986a) Differential regulation by LH and prostaglandins of steroidogenesis in small and large luteal cells of the ewe. *J. Reprod. Fert.* **76**, 821–829.
- Schwall, R.H., Gamboni, F., Mayan, M.H. & Niswender, G.D. (1986b) Changes in the distribution of sizes of ovine luteal cells during the oestrous cycle. *Biol. Reprod.* **34**, 911–918.
- Silvia, W.J., Fitz, T.A., Mayan, M.H. & Niswender, G.D. (1984) Cellular and molecular mechanisms involved in luteolysis and maternal recognition of pregnancy in the ewe. *Anim. Reprod. Sci.* **7**, 57–74.
- Theodosis, D.T., Wooding, F.B.P., Sheldrick, E.L. & Flint, A.P.F. (1986) Ultrastructural localisation of oxytocin and neurophysin in the ovine corpus luteum. *Cell Tissue Res.* **243**, 129–135.
- Ursely, J. & Leymarie, P. (1979) Varying response to luteinizing hormone of two luteal cell types isolated from the bovine corpus luteum. *J. Endocr.* **83**, 303–310.
- Wathes, D.C., Swann, R.W., Birkett, S.D., Porter, D.G. & Pickering, B.T. (1983) Characterization of oxytocin, vasopressin and neurophysin from the bovine corpus luteum. *Endocrinology* **113**, 693–697.
- Wilkinson, R.F., Anderson, E. & Aalberg, J. (1976) Cytological observations of dissociated rat corpus luteum. *J. Ultrastruct. Res.* **57**, 168–184.

Received 7 July 1986