Do small and large luteal cells of the sheep interact in the production of progesterone?

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Summary. Corpora lutea from cyclic ewes were dissociated by collagenase and trypsin/EGTA treatments, and enriched fractions of small and large luteal cells were prepared on gradients of Ficoll. These fractions were incubated separately or remixed before incubation. Colchicine, cytochalasin B and the calcium channel-blocker verapamil significantly reduced progesterone production by both small and large luteal cell fractions, while isoprenaline stimulated an increase in progesterone production by large luteal cell fractions only. When fractions of small and large luteal cells were remixed, no more and no less progesterone was produced than would have been predicted from equivalent fractions incubated separately. There was therefore no evidence of synergism between small and large luteal cells in the production of progesterone. Prostaglandin F-2 α , which can inhibit LH-stimulated progesterone production by ovine luteal tissue *in vitro*, had no effect on LH-stimulated progesterone production by small luteal cell fractions, but significantly inhibited that by enriched fractions of large luteal cells. Since large luteal cell fractions were contaminated with small luteal cells, which are probably responsible for the progesterone-secretory response of these fractions to LH, it was concluded that the inhibition of LH-stimulated progesterone production by small luteal cells is dependent on the presence of large luteal cells. Oxytocin added to large and small luteal cell fractions did not affect progesterone production by either fraction. It was therefore concluded that the inhibitory action of PGF-2 α on LH-stimulated progesterone production may require the interaction of large and small luteal cells, but that oxytocin is not likely to be an intermediary in this interaction.

Introduction

The corpus luteum (CL) of the sheep, like that of several other species, contains large and small luteal cells (Mossman & Duke, 1973). Available evidence suggests that these are distinct cell types, differing in many respects other than size (O'Shea, Cran & Hay, 1979), and that they are probably derived from granulosa and theca cells of ovarian follicles respectively (O'Shea *et al.*, 1980). In-vitro studies have shown that both of these cell types can synthesize and secrete progesterone, although it would appear that only the small luteal cells are able to respond to LH by an increase in progesterone synthesis (Fitz, Mayan, Sawyer & Niswender, 1982; Rodgers & O'Shea, 1982; Rodgers, O'Shea & Findlay, 1983a). No other steroid hormones are known to be produced in significant quantities by the ovine CL, and it is not obvious why two types of progesterone-synthetic cell are required in this species.

One possible explanation for the occurrence of two types of luteal cell in sheep is that, in spite of possessing a shared steroidogenic function, they also serve distinctive functions. Some evidence in

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support of this view is found in the demonstration that oxytocin, secreted by the cyclic CL of ewes (see Wathes, 1984), is produced exclusively by the large luteal cells (Rodgers, O'Shea, Findlay, Flint & Sheldrick, 1983b).

A second possibility is that the large and small luteal cells interact in some way with one another in the production of progesterone. Evidence in support of this has been provided from studies of large and small luteal cells from the pig (Lemon & Mauleon, 1982), in which synergism in progesterone synthesis between the two cell types was demonstrated *in vitro*. Also, prostaglandin (PG) F-2 α can inhibit the LH-stimulable component of progesterone production by ovine luteal tissue *in vitro* (Evrard, Leboulleux & Hermier, 1978; Evrard-Herouard *et al.*, 1981; Fletcher & Niswender, 1982). Since the response to LH appears to be a function only of the small luteal cells, while receptors for PGF-2 α are found primarily on the large luteal cells (Fitz *et al.*, 1982), a cell-to-cell inhibitory interaction is possible. Oxytocin may play an intermediary role in such an interaction, since it is produced by large luteal cells (Rodgers *et al.*, 1983b), is released in response to a PGF-2 α analogue (Flint & Sheldrick, 1982), and is able to alter progesterone production *in vitro* by bovine (Tan, Tweedale & Biggs, 1982a) and human (Tan *et al.*, 1982b) luteal cells.

This paper reports a study to investigate aspects of these two possibilities.

Materials and Methods

Purification and incubation of cells. The method for preparing fractions of small and large luteal cells from ovine CL has been reported in detail by Rodgers & O'Shea (1982). Briefly, ovaries were obtained from cyclic ewes within 15 min of death and transported to the laboratory. Fully developed CL, which showed no histological signs of regression, were selected. The tissues were initially dissociated in collagenase (400 U/ml; Type CLS 1: Worthington Biochemical Co., Freehold, NJ, U.S.A.) in Dulbecco's modified Eagle's medium (DMEM; 10 ml/g luteal tissue; Commonwealth Serum Laboratories, Melbourne, Victoria, Australia) and further dissociated into a single cell suspension by treatment with 0.3% trypsin/1 mM-ethyleneglycol-bis-(β -aminoethyl-ether) *N,N'*-tetra-acetic acid (EGTA). The incubation was terminated by the addition of soybean trypsin inhibitor.

Enriched fractions of small and large luteal cells were prepared on a stepped gradient of Ficoll 400 (Pharmacia Fine Chemicals, Uppsala, Sweden). For each CL, an aliquant of cells from each fraction was counted in a haemocytometer after dilution with trypan blue (Phillips, 1973). The yield of viable cells (those excluding trypan blue) was determined and the cells were classified on the basis of cell diameter. This method of cell classification has been previously validated using electron microscopy to identify the cell types (Rodgers & O'Shea, 1982). Endothelial cells were always contaminants of the luteal cell fractions. Fractions of small luteal cells were relatively free of large luteal cells, but large luteal cell fractions did contain small luteal cells (Table 1). The yield of cells and the degree of purity of each cell fraction varied between CL, hence results were often analysed by pairing the control and treatment results for each CL.

Incubations of cells in DMEM containing 10% fetal calf serum (Flow Laboratories, Inc., McLean, VI, U.S.A.) were carried out for 12 h in quadruplicate in 24-well plates (Flow Laboratories, Inc.) (1 ml per well) at 37°C in a humidified atmosphere of 5% CO₂ in air. Incubations were terminated by freezing (–20°C) the samples.

Hormones and chemicals. Ovine LH (No. 21, biopotency 2.5 $\times$ NIH-LH-S1) was obtained from the National Institute of Arthritis, Diabetes and Digestive and Kidney Disease (Bethesda, MD, U.S.A.). Other hormones and agents were obtained from their respective sources as follows: PGE-2 and PGF-2 α from the Upjohn Co. (Kalamazoo, MI, U.S.A.); LHRH from Peninsula Laboratories (San Carlos, CA, U.S.A.); oxytocin (23.2 i.u./mg) and calcium ionophore A-23187 from Calbiochem Behring Aust. Pty Ltd (Kingsgrove, NSW, Australia); verapamil from Knoll Pharmaceutical

Table 1. The mean composition of the small and large luteal cell fractions from 10 CL used in the present experiments

Cell fraction	% of each luteal cell type*	
	Small luteal cells	Large luteal cells
Small luteal cell	96.2 (94.3–97.7)†	3.8 (2.3–5.7)
Large luteal cell	45.6 (41.4–49.4)	54.4 (50.7–58.6)

*Cells classified according to size in a haemocytometer: small luteal cells 14–19 μ m and large luteal cells > 19 μ m (Rodgers & O'Shea, 1982).

†(Mean minus s.e.m. – mean plus s.e.m.). Determined using the arcsine transformation (Sokal & Rohlf, 1969).

Co. (Whippany, NJ, U.S.A.); and indomethacin, oestradiol-17 β , 5'-guanylimido-diphosphate (Gpp(NH)p), colchicine, cytochalasin B, isoprenaline, N⁶,O^{2'}-dibutyryl adenosine 3',5'-cyclic monophosphate (dibutyryl cAMP), and 1-isobutyl-3-methylxanthine from Sigma Chemical Co. (St Louis, MO, U.S.A.).

Oestradiol-17 β , PGE-2 and PGF-2 α , indomethacin and cytochalasin B were dissolved in ethanol and an aliquant added directly to the incubation medium. The concentration of ethanol in the medium for each of these treatments did not exceed 0.1%. The calcium ionophore A-23187 was initially dissolved in dimethylsulphoxide, which did not exceed 0.01% in the incubation medium.

Progesterone assay. The concentration of progesterone in unextracted samples of appropriately diluted medium in which cells had been previously sonicated was measured by radioimmunoassay (Rodgers *et al.*, 1983a). The antiserum was relatively specific for progesterone, cross-reacting only with 11-hydroxyprogesterone (27%), 11-deoxycorticosterone (6.9%) and corticosterone (5.8%). All other steroids tested, including 20 α -dihydroprogesterone, showed cross-reactivity of < 1.0%. The sensitivity was 6 pg/tube and the intra-assay coefficient of variation was 7.4% at 124 pg/tube (10 assays). The interassay coefficient of variation was 8.7% and 9.5% at 78 and 248 pg/tube respectively. All samples from each CL for each experiment were assayed in the same assay.

Statistical analyses. Data were analysed by paired *t* tests and analysis of variance.

Results

Interactions between small and large luteal cells in the production of progesterone

Fractions of small and large luteal cells were prepared from 5 CL and for each CL the fractions of small luteal cells and large luteal cells were incubated separately and in a 50/50 mixture of the two fractions, with or without the addition of LH (100 ng/ml), dibutyryl cAMP (3 mM plus 0.5 mM-1-isobutyl-3-methylxanthine), oestradiol-17 β (250 ng/ml) or oxytocin (5, 50 or 500 mi.u./ml). These doses of LH and dibutyryl cAMP had previously been shown to stimulate substantially progesterone production by small luteal cells (Rodgers *et al.*, 1983a); the dose of oestradiol-17 β was similar to that found in the fluid of cavities of ovine CL (McNatty, Gibb, Dobson, Thurley & Findlay, 1981), and the doses of oxytocin were based on the concentrations found in ovine CL (Wathes & Swann, 1982; Flint & Sheldrick, 1983). The concentrations of cells from each fraction were arranged so that when the fractions were mixed in equal volumes a predetermined ratio of small luteal cells to large luteal cells was attained. A range of ratios was used, similar to those

observed in whole CL (Rodgers, O'Shea & Bruce, 1984) (one CL each at a small to large luteal cell ratio of 2.5:1, 3:1 and 4:1 and two CL at 5:1). There were no differences in the results between the different CL whose cells were incubated at different ratios, and hence the results from all the CL were pooled and are shown in Text-fig. 1 (data not shown for lower doses of oxytocin). A value of 100% in Text-fig. 1 indicates that there was no synergism or inhibition between the two cell types in the production of progesterone. No more and no less progesterone was produced by recombining the cell types than would have been predicted on the basis of incubations of the cell types separately. This was true for basal and LH-stimulated progesterone, and that produced when dibutyryl cAMP, oestradiol-17 β or oxytocin (5, 50 or 500 mi.u./ml) were present in the medium.

Text-fig. 1. Net amount of progesterone produced (mean $\pm$ s.e.m.) by recombined cell fractions, expressed as a percentage of the average amount of progesterone produced by separated small luteal cell fractions and large luteal cell fractions (5 CL). Incubations were carried out for 12 h in incubation medium alone (basal) or with LH (100 ng/ml), dibutyryl cAMP (dbcAMP; 3 mM in the presence of 0.5 mM-1-isobutyl-3-methylxanthine), oestradiol-17 β (E₂; 250 ng/ml), or oxytocin (500 mi.u./ml). The mean $\pm$ s.e.m. basal progesterone production by small and large luteal cell fractions incubated separately was, respectively, 33.7 ± 6.4 and 269.2 ± 39.7 ng/10⁴ luteal cells over 12 h.

Effects of prostaglandins on progesterone production

Small and large luteal cell fractions were incubated with PGE-2 (50 ng/ml), PGF-2 α (50 ng/ml), or indomethacin (10 μ g/ml) (5 CL) and progesterone production was determined (Text-fig. 2). The production of progesterone by controls containing ethanol (0.1%) was not significantly different from the values with medium only. PGE-2 and PGF-2 α had no significant effect on basal progesterone production by small or large luteal cells. However, PGF-2 α significantly inhibited the LH-stimulated component of progesterone production by large luteal cell fractions ($P < 0.05$), but had no effect on LH-stimulated progesterone production by small luteal cell fractions. Indomethacin had no effect on LH-stimulated progesterone production by either cell fraction.

Effects of oxytocin on progesterone production

Enriched fractions of small and large luteal cells were incubated with oxytocin (0, 5, 50, 500 mi.u./ml) alone or with LH (100 ng/ml) or dibutyryl cAMP (3 mM plus 0.5 mM-1-isobutyl-3-methylxanthine) (5 CL). Progesterone production was not affected by oxytocin at any of the doses tested ($P > 0.05$, analysis of variance) (Text-fig. 3). The oxytocin was biologically active before and after incubation with small or large luteal cells. At a dose of 0.5 mi.u./ml, oxytocin stimulated contractions *in vitro* of a strip of uterus taken from an oestrogen-primed mouse (data not shown). Incubation medium in which small or large luteal cells had been incubated stimulated uterine contractions only if the medium contained oxytocin (10 μ l medium containing 5.0 mi.u. added to a 10 ml organ bath).

Text-fig. 2. Net progesterone production (mean $\pm$ s.e.m.), expressed as a percentage of basal production (100%), by (a) small and (b) large luteal cell fractions incubated for 12 h in the presence of PGE-2 (50 ng/ml), PGF-2 α (50 ng/ml), LH (100 ng/ml) with or without PGF-2 α , or indomethacin (10 μ g/ml). The mean $\pm$ s.e.m. basal progesterone production by small and large luteal cell fractions respectively was 57.1 ± 9.5 and 343.4 ± 95.0 ng/ 10^4 luteal cells over 12 h and 61.2 ± 8.4 and 343.0 ± 98.0 ng/ 10^4 luteal cells over 12 h when ethanol (0.1%) was present in the medium (5 CL). * $P < 0.05$, ** $P < 0.01$ compared with the basal control values within cell type.

Text-fig. 3. Net progesterone production (mean $\pm$ s.e.m.), expressed as a percentage of basal production (100%), by (a) small and (b) large luteal cell fractions incubated for 12 h in the presence of oxytocin in medium alone or in medium containing 100 ng LH/ml or 3 mM-dibutyl cAMP plus 0.5 mM-1-isobutyl-3-methylxanthine. The mean $\pm$ s.e.m. basal progesterone production by small and large luteal cell fractions respectively was 33.7 ± 6.4 and 269.2 ± 39.7 ng/ 10^4 luteal cells over 12 h (5 CL).

Text-fig. 4. Net progesterone production (mean $\pm$ s.e.m.), expressed as a percentage of basal production (100%), by (a) small and (b) large luteal cell fractions incubated for 12 h in the presence of a GTP analogue, 5'-guanylimidodiphosphate (Gpp(NH)p), at 100 μ M, LHRH (1 μ g/ml), oestradiol-17 β (E₂; 250 ng/ml) with or without indomethacin (10 μ g/ml) or isoprenaline (10 μ M). The mean $\pm$ s.e.m. basal progesterone production by small and large luteal cell fractions respectively was 57.1 ± 9.5 and 343.4 ± 95.0 ng/10⁴ luteal cells over 12 h and 61.2 ± 8.4 and 343.0 ± 98.0 ng/10⁴ luteal cells over 12 h when ethanol (0.1%) was present in the medium (5 CL). * $P < 0.05$ compared with basal values within cell type.

Text-fig. 5. Net progesterone production (mean $\pm$ s.e.m.), expressed as a percentage of basal production (100%) by (a) small and (b) large luteal cell fractions incubated for 12 h in the presence of calcium ionophore A-23187 (0.1 μ M), verapamil (100 μ M), colchicine (10 μ M) or cytochalasin B (5 μ g/ml). The mean $\pm$ s.e.m. basal progesterone production by small and large luteal cell fractions respectively was 48.2 ± 8.1 and 360.0 ± 98.9 ng/10⁴ luteal cells over 12 h (5 CL). * $P < 0.05$, ** $P < 0.01$ compared with basal control values within cell type.

Differential responses of small and large luteal cells to various agents

The analogue of GTP, 5'-guanylimidodiphosphate (100 μ M), which in many instances can activate adenylate cyclase directly (see Ross & Gilman, 1980), and LHRH, which has been shown to alter gonadal steroidogenesis (see Sharpe, 1982), did not significantly alter progesterone production by small or large luteal cells (5 CL; $P > 0.05$, paired t tests) (see Text-fig. 4). Oestradiol-17 β , at a dose of 250 ng/ml, added alone or with indomethacin (10 μ g/ml) did not significantly ($P > 0.05$, paired t tests) alter progesterone production (Text-fig. 4). However, isoprenaline, a β -adrenergic agonist, significantly increased progesterone production by large luteal cells but not by small luteal cells ($P < 0.05$, paired t tests).

Small and large luteal cells from the same CL as above were incubated for 2 h in DMEM containing 10% fetal calf serum (0.5 ml) during which time the cells had adhered to the bottom of the well. Treatment and control media (0.5 ml) were then added, and the incubation continued for a further 10 h. Progesterone production was determined (see Text-fig. 5). Calcium ionophore at the low dose of 0.1 μ M did not significantly alter progesterone production by either cell type ($P > 0.05$, paired t tests). However, verapamil (100 μ M), a calcium channel blocker (Singh, Ellruidt & Peter, 1978), significantly inhibited progesterone production by both cell types ($P < 0.05$, paired t tests). Cytochalasin B, an inhibitor of microfilaments (Wessells *et al.*, 1971; Carter, 1972), significantly reduced progesterone production by both cell types. Colchicine, which can cause depolymerization of the tubulin component of microtubules (Olmsted & Borisy, 1973; Wilson, Bamburg, Mizel, Grisham & Creswell, 1974), significantly reduced progesterone production by both cell types ($P < 0.05$, paired t tests).

Discussion

The present results on progesterone production *in vitro* by ovine luteal cells provide no evidence of synergism between the small and large luteal cells nor of a direct role for oxytocin in the control of luteal progesterone production. The results do, however, suggest that the inhibitory action of PGF-2 α on LH-stimulated progesterone production, a function of the small luteal cells, may be mediated via the large luteal cells, and that calcium fluxes, microfilaments and microtubules may influence progesterone production by both cell types. Furthermore, isoprenaline has been identified as an agent capable of stimulating progesterone production by the large luteal cell fractions.

In a study on populations of small and large luteal cells from pigs, Lemon & Mauleon (1982) found that the superfusate from the small luteal cells could increase progesterone production by the large luteal cells, but not *vice versa*. However, Ursely & Leymarie (1979) did not observe any such synergism using bovine luteal cells. Under the conditions in the present experiments, no enhancement of progesterone production was observed when small and large luteal cell fractions were mixed, even in the presence of LH, dibutyryl cAMP, oestradiol-17 β or oxytocin in the medium. The possibility that small luteal cells can stimulate progesterone production by large luteal cells cannot be ruled out: the *in-vitro* conditions used may not have been sufficiently optimal for synergism to occur and such synergism could occur *in vivo*. It is also possible that the degree of small luteal cell contamination of the large luteal cell fractions may have been sufficient to negate any effect of further addition of small luteal cells. On the other hand, since the small luteal cell fractions were extremely pure (mean ratio of small luteal cells to large luteal cells of 49:1) and since LH-stimulated progesterone production is a function of small luteal cells (Rodgers *et al.*, 1983a), it can be concluded that large luteal cells did not enhance LH-stimulated progesterone production by the small luteal cells.

Prostaglandin F-2 α is considered to be the uterine luteolysin in the ewe (see Goding, 1974; Horton & Poyser, 1976; Niswender, 1981), although the mechanism by which it exerts its luteolytic effect is still not yet clear. PGF-2 α can inhibit progesterone secretion and luteal blood flow *in vivo*

(Niswender, Reimers, Diekman & Nett, 1976) and can inhibit LH-stimulated, but apparently not basal, secretion of progesterone by ovine luteal tissue *in vitro* (Evrard *et al.*, 1978; Evrard-Herouard *et al.*, 1981; Fletcher & Niswender, 1982). In the present *in-vitro* experiments, PGF-2 α inhibited only LH-stimulated progesterone production by the enriched fractions of large luteal cells, but not that by the very LH-responsive small luteal cell fractions. Fitz *et al.* (1982) and Rodgers *et al.* (1983a) considered that the LH-stimulated component of progesterone production by the large luteal cell fractions is due to the contaminating small luteal cells in these fractions. It would therefore appear that the inhibition of LH-stimulated progesterone production by the small luteal cells is dependent on the presence of large luteal cells. Such an interaction of this type between the two cell types could be effected by the location of the LH receptors on small luteal cells and PG receptors on large luteal cells (Fitz *et al.*, 1982), and could partly explain why there are two types of cells in the sheep CL.

Studies showing deleterious effects of PGF-2 α on large luteal cells in culture (Fitz, Mock, Mayan & Niswender, 1984) indicate other interpretations of the present *in-vitro* results. It is possible that PGF-2 α in the present experiments also had such deleterious effects and caused the observed inhibition of progesterone production by fractions of large luteal cells. However, this would not explain why PGF-2 α had no effect on progesterone production by fractions of small luteal cells in the present experiments. In earlier experiments PGF-2 α inhibited LH-stimulable cAMP and progesterone production (Evrard *et al.*, 1978; Evrard-Herouard *et al.*, 1981; Fletcher & Niswender, 1982), both functions of small luteal cells (Fitz *et al.*, 1982; Rodgers & O'Shea, 1982; Rodgers *et al.*, 1983a; Hoyer, Fitz & Niswender, 1984). These inhibitory effects of PGF-2 α were detected by use of tissue slices, in which small and large luteal cells are present. This also suggests that PGF-2 α inhibition of LH-stimulable progesterone production is dependent on the presence of large luteal cells.

One mechanism by which PGF-2 α could exert its inhibitory effect via the large luteal cells is for it to stimulate the release of an inhibitory substance from the large luteal cells which in turn could act on the small luteal cells. Oxytocin was a prime candidate for this role, having been shown to be produced by the large luteal cells (Rodgers *et al.*, 1983b), released *in vivo* in response to PGF-2 α administration (Flint & Sheldrick, 1982), and able to alter progesterone production *in vitro* by bovine and human CL (Tan *et al.*, 1982a, b). However, the results of the present experiment showed that biologically-active oxytocin had no effect on progesterone production by either luteal cell type from the sheep. Furthermore, Flint & Sheldrick (1983) have suggested that if oxytocin is involved in luteolysis it is more likely to be via a systemic than an intraluteal route because hysterectomy prevents the luteolytic action of oxytocin in cattle and cloprostenol is luteolytic in ewes after hysterectomy, when luteal oxytocin concentrations are low (Sheldrick & Flint, 1983). Oxytocin therefore does not appear to mediate the inhibitory action of PGF-2 α on the progesterone production that occurs *in vitro*.

In the present experiments indomethacin, PGE-2, LHRH, oestradiol-17 β and 5'-guanylimidodiphosphate did not have any significant effect on progesterone production by either cell type. Calcium ionophore, which at high doses (5 μ M) has been shown to stimulate a small (30%) increase in progesterone production by slices of ovine corpora lutea (Sawyer, Abel, McClellan, Schmitz & Niswender, 1979), did not significantly alter progesterone production when used at low doses in the present experiments. However, verapamil, cytocholasin B and colchicine did inhibit progesterone production by both luteal cell types, suggesting that, if these inhibitors are acting specifically, the production of progesterone by both luteal cell types is dependent upon calcium fluxes, microfilaments and microtubules.

Colchicine causes depolymerization of the tubulin component of microtubules (Olmsted & Borisy, 1973; Wilson *et al.*, 1974) and thus inhibits the action of microtubules. Inhibitors of microtubules have previously been shown to reduce ovarian steroidogenesis (Gemmell & Stacy, 1977; Sawyer *et al.*, 1979; Gwynne & Condon, 1982; Azhar & Menon, 1981; Tsang & Carnegie, 1983). However, the role of microtubules in steroidogenesis is unclear. Colchicine has been shown

to disrupt the formation (Gemmell & Stacy, 1977; Sawyer *et al.*, 1979) and secretion (Gemmell & Stacy, 1977) of 0.2 µm membrane-bound secretory granules from the ovine large luteal cell and at the same time reduce progesterone secretion. Since these granules have been postulated to contain progesterone (Gemmell, Stacy & Thorburn, 1974; Sawyer *et al.*, 1979) it was proposed that colchicine inhibition of granule formation was one mechanism by which colchicine reduced progesterone production (Gemmell & Stacy, 1977; Sawyer *et al.*, 1979). In the present study progesterone production by small luteal cells, which have not been observed to secrete the contents of 0.2 µm granules (O'Shea *et al.*, 1979), was reduced by colchicine. This suggests that the mode of action of colchicine on progesterone production is not necessarily via inhibition of granule formation and indirectly it suggests that granule formation is unlikely to be related to progesterone production.

Isoprenaline, a β-agonist, stimulated a significant, but small (50%), increase in progesterone production by the large luteal cell fractions but not by the small luteal cell fractions. This suggests that the initial action of isoprenaline was on the large luteal cells. β-Adrenergic compounds have been shown to activate the adenylate cyclase enzyme of CL of a number of species (ox: Godkin, Black & Duby, 1977; sheep: Jordan, Caffrey & Niswender, 1978; rat: Ratner, Weiss & Sanborn, 1980; rabbit: Abramowitz & Birnbaumer, 1982), suggesting that cAMP may be the second messenger in the action of β-adrenergic compounds. Ovine large luteal cells have an adenylate cyclase, which although unresponsive to LH, can be activated directly by forskolin (Hoyer *et al.*, 1984). Under forskolin stimulation (Hoyer *et al.*, 1984) and in the presence of dibutyryl cAMP (Rodgers *et al.*, 1983a), small increases in progesterone production, similar to that seen here with isoprenaline, have been observed.

In conclusion, no evidence was found of any synergistic interaction between the small and large luteal cells of sheep. However, the results suggest that the action of PGF-2α on LH-stimulated progesterone secretion involves a cell-to-cell inhibitory action that appears not to be mediated via oxytocin.

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