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Intraovarian regulation of luteolysis

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The corpus luteum is a transient gland, which is only functional for 17–18 days in the cyclic cow or for up to 200 days in the pregnant cow. Regression of the corpus luteum is essential for normal cyclicity as it allows the development of a new ovulatory follicle, whereas prevention of luteolysis is necessary for the maintenance of pregnancy. Evidence acquired over the past three decades indicated that $PGF_{2\alpha}$ is the luteolytic hormone in ruminants. Nevertheless, the detailed mechanisms of $PGF_{2\alpha}$ action are just beginning to be clarified. A pivotal role for an endothelial cell product endothelin 1 (ET-1) has been documented in $PGF_{2\alpha}$-induced luteal regression. ET-1 inhibited progesterone production by luteal cells in a dose-dependent manner via selective ET-1 binding sites (ET_A). The inhibitory action of $PGF_{2\alpha}$ on progesterone secretion (*in vivo* and *in vitro*) was blocked by a selective ET_A receptor antagonist. This implied that ET-1 (through ET_A receptors present on steroidogenic cells) may have mediated the inhibitory effect of $PGF_{2\alpha}$. The involvement of ET-1 in luteal regression was also suggested by the observation that the highest concentrations of ET-1 coincide with uterine $PGF_{2\alpha}$ surges. Furthermore, $PGF_{2\alpha}$ administration upregulated ET-1 expression within the corpus luteum. Later stages of luteal regression, which involve programmed cell death (PCD), are presumably mediated by immune cells. ET-1 may also be involved in this process by promoting leukocyte migration and stimulating macrophages to release tumour necrosis factor α (TNFα). The TNFα receptor type 1 (p55) is present on luteal cells (endothelial and steroidogenic cells) and could initiate PCD and the structural demise of the corpus luteum.

Introduction

In cattle and other species, the corpus luteum plays a central role in the regulation of cyclicity and in the maintenance of pregnancy (Hansel and Blair, 1996). This gland undergoes dynamic changes throughout its life span; its formation, induced by the luteotrophic hormone LH, involves a complex process of cell differentiation and neovascularization (Jablonka-Shariff *et al.*, 1993; Fields and Fields, 1996). This highly vasculated endocrine organ functions to prepare the female reproductive system to receive and maintain a conceptus. In the absence of an embryonic signal, the corpus luteum will regress. Since the early 1970s substantial evidence has accumulated to indicate that the primary luteolysin in domestic ruminants is $PGF_{2\alpha}$ of uterine origin (McCracken *et al.*, 1972; Ellinwood *et al.*, 1979; Pate, 1994).

Administration of $PGF_{2\alpha}$ between day 5 and day 15 of the bovine oestrous cycle triggers a sequence of irreversible changes in the corpus luteum, similar to the spontaneously occurring events (McCracken *et al.*, 1972; Pate, 1994). Initially, there is reduction in progesterone release, and later an infiltration of macrophages and morphological changes consistent with apoptotic cell death (Juengel *et al.*, 1993; Hahnke *et al.*, 1994). Corpus luteum regression is necessary for normal cyclicity, as a functional corpus luteum suppresses the final stages of follicular development which leads to

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ovulation. Not only is the suppression of corpus luteum function (that is progesterone production) required, but the gland must also be physically eliminated, to keep the ovary at its proper size. Luteal regression may be regarded as consisting of two processes – functional and structural luteolysis – that differ in their temporal and mechanistic features. Functional luteolysis refers to the rapid decline in luteal progesterone, while structural luteolysis describes the events leading to the structural demise of the corpus luteum and is accomplished within a few days.

In contrast to the well documented consistent luteolytic effects of $PGF_{2\alpha}$ *in vivo*, studies examining its direct effects on luteal cells produced controversial data, especially when using isolated luteal cell populations in which it had paradoxically increased progesterone production (Davis *et al.*, 1989; Meidan *et al.*, 1991; Miyamoto *et al.*, 1993). This discrepancy had led us to postulate that a non-steroidogenic cell, such as the endothelial cell, may mediate the actions of $PGF_{2\alpha}$. The possible involvement of endothelial cells in physiology of the corpus luteum is supported by their abundance (> 50% of total cells of the corpus luteum; O'Shea *et al.*, 1989, and their associated contact with steroidogenic cells (Grazul-Bilska *et al.*, 1992). Endothelial cells have the unique ability to sense changes in blood flow, blood pressure and oxygen tension to which they respond by the appropriate upregulation of endothelin 1 (ET-1) expression. As a result of its strategic location, the endothelium layer can integrate a myriad of physical and biochemical signals within an organ; therefore, it is not surprising that recent evidence indicates that these cells are key players in many biological functions (Inagami *et al.*, 1995). At the time of luteal regression, macrophages invade the corpus luteum; these cells might participate in apoptotic events by secreting cytokines such as tumour necrosis factor α (TNFα). Athough the role of TNFα in the regression of the corpus luteum is presently unknown, it should be noted that peak amounts of TNFα within the corpus luteum correspond to the initiation of the apoptotic process (Shaw and Britt, 1995).

This review summarizes studies documenting the involvement of endothelial cells and macrophages in $PGF_{2\alpha}$-induced regression of the corpus luteum.

Role of Endothelial Cells in $PGF_{2\alpha}$-induced Anti-steroidogenic Action

The corpus luteum is a heterogeneous tissue and besides endothelial cells and steroidogenic large luteal (LLC) and small luteal (SLC) cells it also consists of fibroblasts, smooth muscle cells and immune cells (O'Shea *et al.*, 1989). Therefore, two experimental models were used to study the involvement of endothelial cells in the anti-steroidogenic actions of $PGF_{2\alpha}$: (1) luteal slices in which the integrity and communication between the various cells is preserved, and (2) isolated luteal steroidogenic cells co-cultured in the presence of endothelial cells (Girsh *et al.*, 1995).

The accumulation of progesterone secretion from 2- to 4-day-old corpus luteum slices is shown in Fig. 1a. $PGF_{2\alpha}$ (1 μg ml^{-1}) and LH (100 ng ml^{-1}) increased progesterone secretion to values that were 60% higher than control values; however, this increase was not statistically significant (Fig. 1a). Incubation with LH + $PGF_{2\alpha}$ did not alter progesterone secretion in these young corpora luteal slices, in relation to LH (Fig. 1a). In contrast, in mature corpora luteal slices (6- to 12-day-old), LH stimulated progesterone secretion by 1.6 times after incubation for 30 min (11.2 versus 6.7 ng mg^{-1} corpus luteum). The LH-stimulated progesterone curve rose steadily until 5 h of incubation, when it reached concentrations that were 2.8-fold higher than control values (Fig. 1b). In contrast to results with slices obtained from young corpus luteum, $PGF_{2\alpha}$ significantly reduced progesterone stimulation by LH in the mature corpus luteum ($P < 0.02$; Fig. 1b). The finding that the effect of $PGF_{2\alpha}$ on luteal tissue was age dependent is in agreement with other reports demonstrating that $PGF_{2\alpha}$ can induce luteolysis only after day 5 of the cycle (Pate, 1994). When a model with isolated luteal steroidogenic and endothelial cells was used, $PGF_{2\alpha}$ did not inhibit progesterone secretion when incubated with steroidogenic cells; the anti-steroidogenic effect of $PGF_{2\alpha}$ was apparent only when luteal cells were co-cultured together with endothelial cells (Girsh *et al.*, 1995). This was the first indication that the anti-steroidogenic effect of $PGF_{2\alpha}$ may be mediated by factors released from the resident endothelial cells in corpus luteum tissue. On the basis of these findings, we investigated the effects of an endothelial cell product ET-1 on $PGF_{2\alpha}$-induced luteal regression.

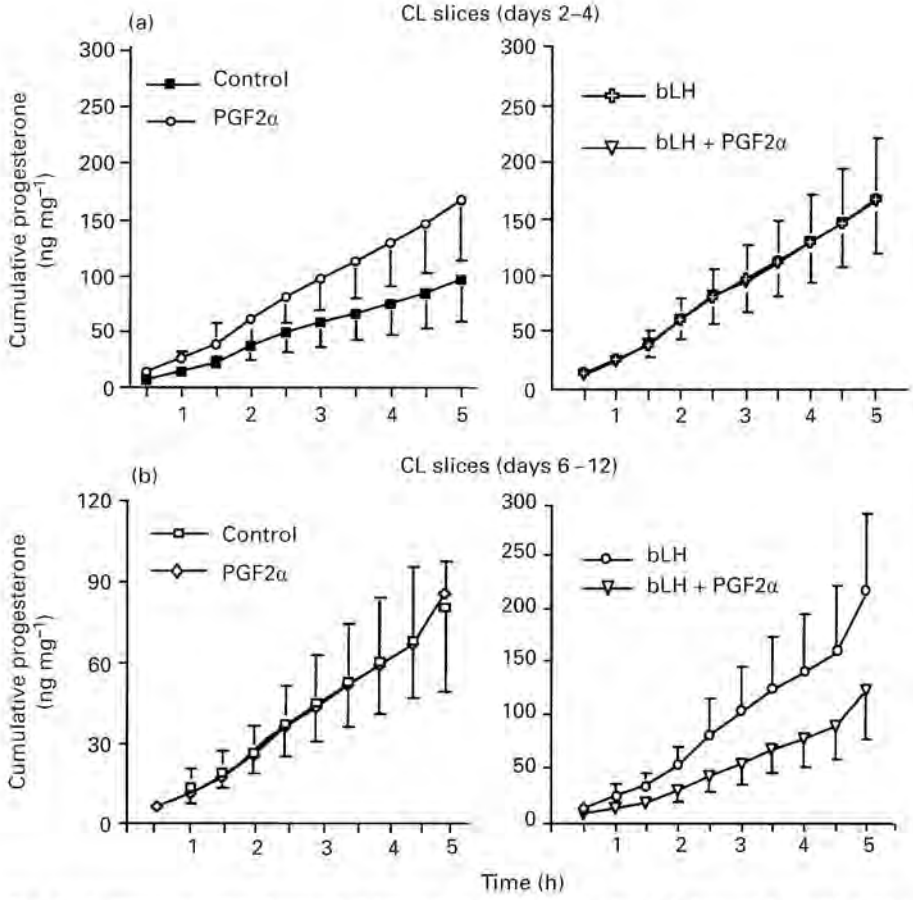

Fig. 1. Cumulative progesterone production (ng mg^{-1} tissue) by 2–4-day-old (a), and 6–12-day-old (b) corpora lutea slices. Slices were preincubated in DMEM/HEPES containing 5% FCS for 2 h at 37.5°C. Slices were then incubated for an additional 5 h in the same media (control) or with the addition of $PGF_{2\alpha}$ (1 μg ml^{-1}), LH (100 ng ml^{-1}) or LH + $PGF_{2\alpha}$. During the preincubation and incubation periods media were replaced every 30 min. Results are the means ± SEM of three corpora lutea.

Inhibition of Luteal Steroidogenesis by ET-1

Endothelin 1 is synthesized and secreted by endothelial cells. This peptide was originally isolated from porcine aortic endothelial cells (Yanagisawa *et al.*, 1988), and subsequently has been found in a wide range of tissues including ovarian granulosa, endometrial and placental (Yanagisawa and Masaki, 1989). ET-1 – a 21 amino acid peptide– is one of the most potent vasoconstrictor peptides known; it belongs to a structurally homologous peptide family which includes ET-2, ET-3 and sarafotoxins (Yanagisawa and Masaki, 1989; Luscher *et al.*, 1992). It is synthesized as a 203 amino acid prepropeptide, which is proteolytically cleaved to produce big ET-1, which is finally processed to the mature form of ET-1 by an endothelin-converting enzyme (Luscher *et al.*, 1992; Opgenorth *et al.*, 1992). Induction of ET-1 secretion results from an enhanced preproET-1 gene expression since ET-1 is not present in storage pools (Yanagisawa and Masaki, 1989; Opgenorth *et al.*, 1992). Although the three ETs are products of three distinct genes, they share extensive sequence homology and a

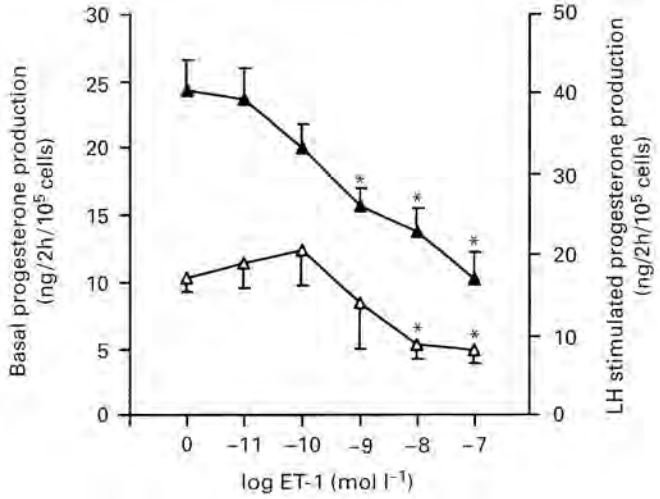

Fig. 2. Dose–response effect of endothelin 1 (ET-1) on progesterone secretion (ng 10^{-5} cells) from dispersed bovine luteal cells. After collagenase dispersion, luteal cells were incubated with various doses of ET-1 in the presence (▲) or absence (△) of bLH (5 ng ml^{-1}; USDA b5). Progesterone concentrations represent the concentration in media after incubation for 2 h. Data are the means ± SEM, $n = 5$, $^*P < 0.05$.

common structural design (Yanagisawa and Masaki, 1989). Vascular endothelial cells produce mainly ET-1, and much less of the other two peptides (Yanagisawa and Masaki, 1989)

The effects of ET-1 on cells derived from the corpus luteum are shown in Fig. 2. Both basal- and bLH (5 ng ml^{-1})-stimulated progesterone secretion from luteal cells were inhibited by ET-1 in a dose dependent manner (Fig. 2; Girsh *et al.*, 1996a). Using an *in vitro* microdialysis system (MDS), Miyamoto *et al.* (1997) demonstrated that $PGF_{2\alpha}$ acutely stimulated the release of progesterone from bovine corpus luteum tissue. Preincubation with $PGF_{2\alpha}$ potentiated the inhibitory action of ET-1 on progesterone production (Miyamoto *et al.*, 1997). Of the two steroidogenic luteal cells, only the granulosa-derived cells responded to this peptide with an inhibition of basal and cAMP-stimulated progesterone production (Girsh *et al.*, 1996a). Similarly, when endothelial–luteal co-cultures were used, there was a ($PGF_{2\alpha}$-induced) reduction in progesterone, which was observed only in the large (granulosa-derived) cells (Girsh *et al.*, 1995). However, in luteal–endothelial co-cultures, the inhibitory effect of $PGF_{2\alpha}$ was attained after a longer incubation. The difference in time scale between these two experimental models may have been due to the time required for endogenous ET-1 to be released before a reduction in progesterone could be observed. The mechanism whereby ET-1 affects steroidogenesis warrants further research.

Expression of ET-1 Receptors in Luteal Cells

Two distinct receptor subtypes of the ET family have been cloned (Arai *et al.*, 1990; Sakurai *et al.*, 1990). These receptors belong to the seven transmembrane G-coupled superfamily and have been termed ET_A (for aorta) and ET_B (for bronchus). The order of potency of the different endothelins binding to the ET_A receptor is ET-1,ET-2 > ET-3 (Arai *et al.*, 1990). The other receptor subtype, ET_B, exhibits equipotent affinity for these three peptides (Sakurai *et al.*, 1990). Endothelin, via binding to these receptors, activates numerous transmembrane signalling systems in various tissues and cell types. The multiple ET-stimulated signal transduction pathways probably contribute to the diversity

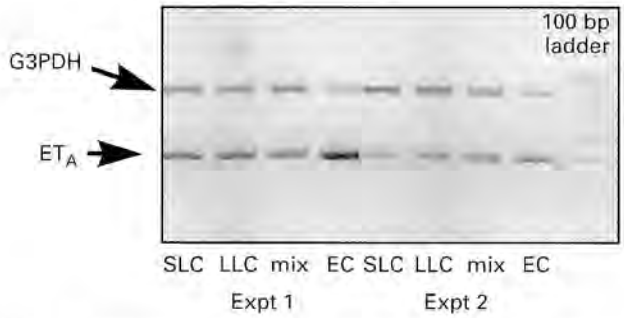

Fig. 3. Expression of endothelin 1 binding sites (ET_A) in bovine luteal cells. Endothelial cells (EC) and small and large luteal cells (SLC and LLC, respectively) were enriched from bovine corpora lutea by elutriation. Mixed cells (mix) – non-separated dispersed cells. Input of 100 ng total RNA of each sample was reverse transcribed and amplified for 21 and 26 cycles (with G3PDH and ET_A primers, respectively). PCR products were subjected to electrophoresis on 2% agarose gel and stained with ethidium bromide. An inverse image is presented.

of the biological responses induced by this peptide. Besides its well described effects on vascular smooth muscle cells and myocytes, ET-1 modulates steroidogenesis in several cell types including ovarian rat granulosa cells. Nevertheless, the identification of ET-1 receptors type(s) expressed by these cells was inconclusive (Iwai *et al.*, 1991; Tedeschi *et al.*, 1992; Iwai *et al.*, 1993; Flores *et al.*, 1995). Saturable, high-affinity ET-1 binding sites were identified in mid-cycle bovine corpus luteum and in the two steroidogenic luteal cell types (Girsh *et al.*, 1996a). These binding sites are of the ET_A subtype as demonstrated by competition with BQ123 (a selective ET_A antagonist) and mRNA expression. ET_A mRNA was detected in SLC, LLC and endothelial cells enriched from bovine corpus luteum (Fig. 3).

In agreement with binding data, ET-1 induced inhibition in progesterone release was prevented when cells were preincubated with a BQ compound. In addition, the addition of ET-3 (up to 10^{-6} mol l^{-1}) was ineffective in terms of progesterone secretion from cells derived from the corpus luteum, either under basal or bLH-stimulated conditions (Girsh *et al.*, 1996a).

The anti-steroidogenic effect of $PGF_{2\alpha}$ was retained in slices of corpus luteum incubated *in vitro* (Girsh *et al.*, 1995). Therefore, the involvement of ET-1 in $PGF_{2\alpha}$-induced progesterone reduction was studied using luteal slices (Girsh *et al.*, 1996a). Incubation with $PGF_{2\alpha}$ significantly inhibited LH-stimulated progesterone secretion (Girsh *et al.*, 1996). When BQ610 was added to the incubation medium it prevented the decline in progesterone secretion. Similarly, a single intraluteal injection of BQ123 to ewes at the mid-luteal phase was sufficient to attenuate the luteolytic action of exogenous $PGF_{2\alpha}$ compared with saline pretreatment (Fig. 4), implying that ET-1 (acting via ET_A receptors) mediated the inhibitory effect of $PGF_{2\alpha}$. The involvement of ET-1 in luteal regression is further suggested by the findings demonstrating that $PGF_{2\alpha}$ could upregulate ET_A expression in small and large luteal-like cells, obtained after *in vitro* luteinization (R. Meidan and N. Levy, unpublished data). However, whether the content of ET-1 receptors in the corpus luteum fluctuates throughout the oestrous cycle is unknown.

Induction of ET-1 Gene Expression by $PGF_{2\alpha}$

The increase in ET-1 production suggested by luteal–endothelial co-cultures (Girsh *et al.*, 1995) can be explained, at least in part, by a direct effect of $PGF_{2\alpha}$ on endothelial cells. Indeed, we found that

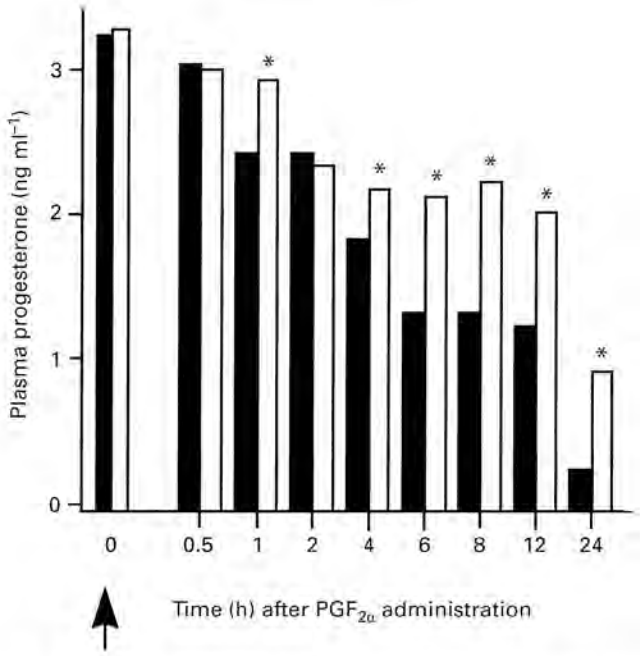

Fig. 4. Effect of endothelin 1 binding site (ET_A) antagonist on plasma progesterone concentrations. Normally cyclic ewes were injected with either saline (control ■) or 100 μg BQ123 (□), 30 min before the administration of 10 mg $PGF_{2\alpha}$ (Lutalyse, i. m.). Ewes underwent flank laparotomy on day 8 or 9 of the oestrous cycle and each corpus luteum per animal was injected with the appropriate treatment. Data are the means ± SEM, $n = 5$. *Significantly different from control ($P < 0.05$).

$PGF_{2\alpha}$ stimulated ET-1 secretion and its mRNA expression in luteal endothelial cells (Girsh *et al.*, 1996b). Under basal conditions the amount of ET-1 produced after incubation for 3 h was 30.2 ± 6.69 pg 10^{-5} cells and the addition of $PGF_{2\alpha}$ (1 μg ml^{-1}) increased ET-1 production approximately threefold. $PGF_{2\alpha}$ induced ET-1 expression in a time-dependent manner. An initial rise in ET-1 mRNA was detected within 15 min after addition of $PGF_{2\alpha}$ and ET-1 mRNA content continued to rise and reached a value 2.5 times higher than those of non-treated cells after 3 h (Girsh *et al.*, 1996). These effects are exerted via specific $PGF_{2\alpha}$ receptors (of the FP type), expressed by luteal endothelial cells (Mamluk *et al.*, 1998). This study was the first to report the presence of $PGF_{2\alpha}$ receptors in luteal endothelial cells.

These data imply that, at least under *in vitro* conditions, $PGF_{2\alpha}$ can rapidly enhance ET-1 expression. However, for ET-1 to have a physiological role in luteal regression, ET-1 concentrations should be high during this phase of the cycle and should vary in a $PGF_{2\alpha}$-dependent fashion.

ET-1 and ET-1 mRNA content were determined in corpus luteum obtained at different stages of the bovine oestrous cycle (Girsh *et al.*, 1996b). ET-1 content was highest in corpus luteum collected on days 17–21 of the cycle (30 times higher than on days 5–6). Similarly, on day 18 there was an increase in ET-1 mRNA expression compared with days 5 and 10 of the oestrous cycle (Fig. 5; Girsh *et al.*, 1996b). Ohtani *et al.* (1998) also observed that highest ET-1 concentrations were found in peripheral plasma during luteolysis. These findings suggest that enhancement of ET-1 expression in the aged corpus luteum could result from uterine $PGF_{2\alpha}$ secretion which occurs at this stage of the bovine cycle. This contention was examined by injecting a luteolytic dose of $PGF_{2\alpha}$ into heifers during mid-

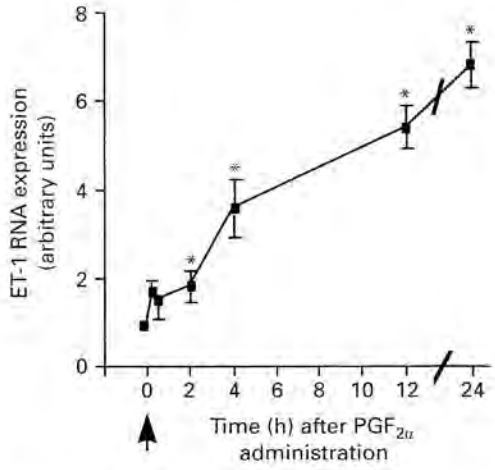

Fig. 5. Endothelin 1 (ET-1) mRNA expression after administration of $PGF_{2\alpha}$ to heifers on day 10 of the oestrous cycle. Corpora lutea were collected at various times after administration of $PGF_{2\alpha}$ (25 mg Lutalyse) as indicated. Data (densitometric units) were normalized to the value of the untreated control (day 10 corpora lutea). Data are means ± SEM from three corpora lutea at each time point. *Significantly different from control ($P < 0.05$).

cycle, resulting in a marked increase in the expression of ET-1 (Fig. 5). Likewise, incubation of luteal slices with $PGF_{2\alpha}$ resulted in a four-fold increase in their ET-1 concentrations (Girsh *et al.*, 1996b). These findings have been corroborated and extended by Miyamoto *et al.* (1997), who reported that $PGF_{2\alpha}$ significantly stimulated release of ET-1 from corpus luteum pieces *in vitro* after infusion for 2 h. Ohtani *et al.* (1998) have documented real time, intraluteal changes in ET-1, oxytocin and progesterone with an MDS device implanted *in vivo*. After administration of $PGF_{2\alpha}$ there was a rapid increase in ET-1 and oxytocin which was accompanied by simultaneous inhibition in progesterone produced by luteal tissue *in vivo*.

Clearly, these data show that $PGF_{2\alpha}$, both under *in vitro* and *in vivo* conditions, quickly augments luteal expression of ET-1 mRNA and protein content.

Role of Endothelial Cells and ET-1 in Structural Luteolysis

ET-1 may also be involved in later stages of luteal regression – namely in structural luteolysis. At present this is a poorly understood process, which is characterized by atrophy of corpus luteum tissue followed by the formation of scar tissue (Fields and Fields, 1996). Atrophy of corpus luteum tissue, which includes both vascular and endocrine epithelial cells, is a selective process involving programmed cell death (PCD) (Juengel *et al.*, 1993; Shikone *et al.*, 1996). Structural luteolysis is also accompanied by the influx of leukocytes, mainly macrophages (Hahnke *et al.*, 1994) and the local secretion or expression of several inflammatory cytokines, such as TNFα (Shaw and Britt, 1995; Wuttke *et al.*, 1997) and monocyte chemoattractant protein 1 (MCP-1) (Tsai *et al.*, 1997; Haworth *et al.*, 1998). Migration of leukocytes is a complex process that involves direct interaction of the migrating cells with endothelium (Mantovani *et al.*, 1997). Thus, any process leading to recruitment of

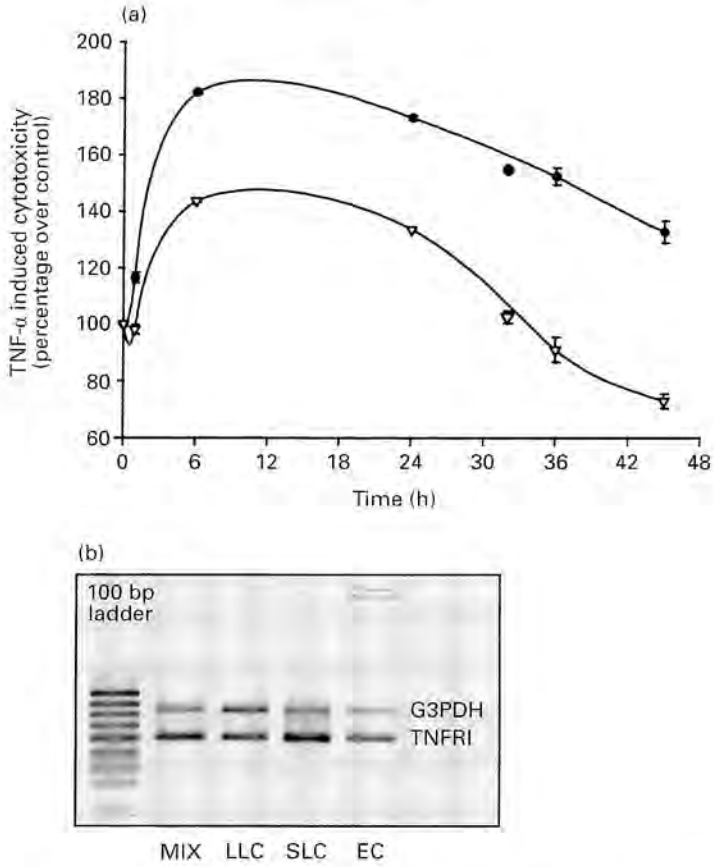

Fig. 6. (a) Peripheral blood derived bovine macrophages were cultured in the presence of increasing doses of endothelin 1 (ET-1) (● 10^{-7} mol ET-1 l^{-1}; ▽ 10^{-9} mol ET-1 l^{-1}). Supernatants were collected at different time intervals and assayed for tumour necrosis factor α (TNFα) activity in a bioassay using a TNFα sensitive WEHI cell line. (b) Expression of p55 TNFR1 in bovine luteal cells. Endothelial cells (EC), small and large luteal cells (SLC and LLC, respectively) were enriched from bovine corpora lutea by elutriation. Mixed cells (MIX) are non-separated dispersed cells. Input of 100 ng total RNA of each sample was reverse transcribed and amplified for 23 and 28 cycles (with G3PDH and TNFR1 primers, respectively). PCR products were subjected to electrophoresis on a 2% agarose gel and stained with ethidium bromide. An inverse image is presented.

leukocytes to the corpus luteum would have to involve endothelial cells. Such a possibility is indicated by our observations that, in response to $PGF_{2\alpha}$ stimulation, corpus luteum-derived endothelial cells secrete ET-1, a cytokine previously shown to be important for leukocyte migration (Boros *et al.*, 1998). To establish further a functional link between macrophages, endothelium and PCD in the corpus luteum, we determined responses of macrophages to endothelial cytokines (ET-1) and investigated expression of receptors for TNFα in different cell populations of the bovine corpus luteum.

ET-1 induced secretion of TNFα by bovine macrophages: 10^{-7} mol l^{-1} was more effective than 10^{-9} mol l^{-1}, and peak secretion was measured between 6 and 24 h of incubation (Fig. 6a). Dispersed

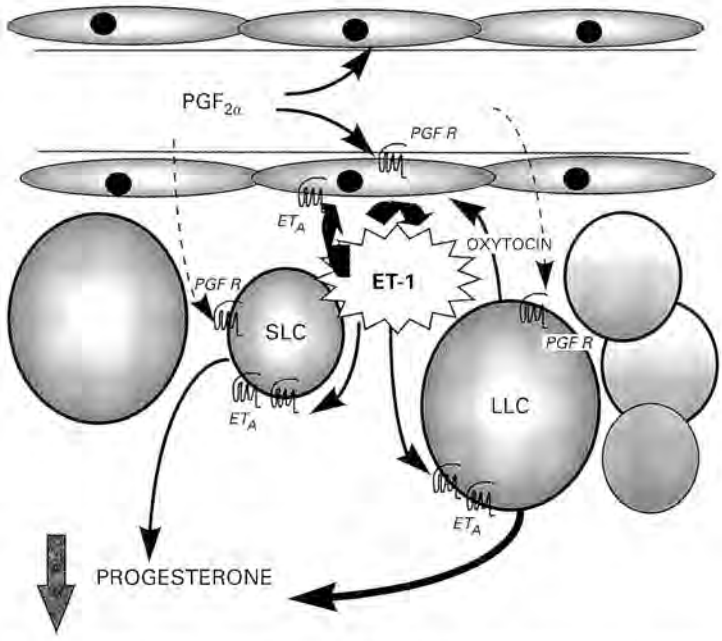

Fig. 7. Model of steroidogenic–endothelial cell interactions during functional luteolysis in ruminants. $PGF_{2\alpha}$ directly enhances endothelin 1 (ET-1) production by the resident endothelial cells, acting through the FP type receptor present in these cells. Large luteal cells may also respond to $PGF_{2\alpha}$ by secreting oxytocin, and increasing ET-1 expression. ET-1 is preferentially released towards the basal cell surface rather than towards the apical surface, and may thus reach nearby luteal steroidogenic cells to reduce their progesterone output. $PGF_{2\alpha}$ and ET-1 both induce vasoconstriction in the corpus luteum, and subsequently hypoxic conditions may develop and further augment ET-1 secretion via a positive feedback loop. ET_A: type A ET-1 receptor; PGFR: $PGF_{2\alpha}$ receptor; SLC: small luteal cells; LLC: large luteal cells.

total corpus luteum cells, SLC, LLC and endothelial cells express the p55 type receptor for TNFα (receptor for secreted form TNFα (Mantovani *et al.*, 1997); Fig. 6b).

Collectively, these results indicate that endothelial cells might have a pivotal role in structural luteolysis via $PGF_{2\alpha}$-induced secretion of ET-1. This statement is supported by data showing high MCP-1 in the corpus luteum in response to $PGF_{2\alpha}$ (Tsai *et al.*, 1997; Haworth *et al.*, 1998), and although the cells secreting MCP-1 were not identified in these studies, previous investigations showed that endothelial cells are a source of MCP-1 (Mukaida *et al.*, 1992).

Conclusions

We demonstrated that $PGF_{2\alpha}$ stimulates luteal ET-1 production by several mechanisms that are not mutually exclusive (summarized in Fig. 7). Large luteal cells respond to $PGF_{2\alpha}$ by secreting oxytocin, which could increase ET-1 production by resident endothelial cells. $PGF_{2\alpha}$ can also directly enhance ET-1 production in endothelial cells, acting through the FP type receptor present in these cells. ETs are preferentially released towards the basal cell surface rather than towards the apical surface, and may thus reach nearby luteal steroidogenic cells and reduce their progesterone output. In addition,

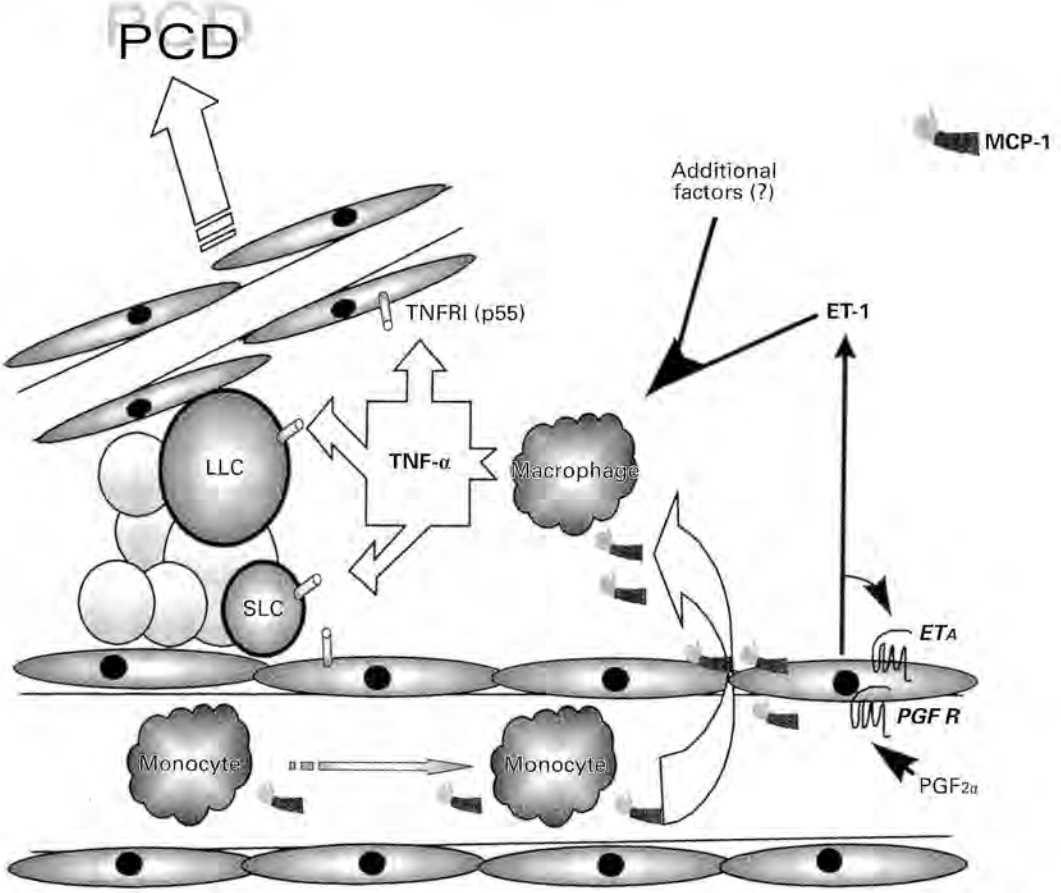

Fig. 8. Model for structural regression of the corpus luteum of ruminants. Conditions established during functional regression, that is endothelial cell activation and cytokine secretion, form the initial phase of structural regression, which assigns a key role to endothelium and macrophages in events leading to programmed cell death (PCD) in the corpus luteum. $PGF_{2\alpha}$ sensitized endothelial cells might initiate a cascade of events leading to the recruitment and transmigration of monocytes to the corpus luteum. (The model proposes that monocyte chemoattractant protein 1 (MCP)-1 is the inducer of migration). Migration and concomitant maturation to macrophages is followed by their activation, which could lead to PCD via secretion of tumour necrosis factor α (TNFα). ET-1: endothelin 1; TNFR1: receptor type 1 (p55) for TNFα

$PGF_{2\alpha}$ may potentiate the inhibitory effect of ET-1 on progesterone release. $PGF_{2\alpha}$, like ET-1 but somewhat weaker, can induce constriction of smooth muscle cells in arterioles. Subsequent to vasoconstriction, hypoxic conditions may develop and further augment ET-1 secretion via a positive feedback loop. The existence of multiple pathways may be instrumental in ensuring the marked increase in luteal endothelin secretion. The quick upregulation of ET-1 mRNA transcription in luteal endothelial cells coupled with lack of peptide storage pools, a unique feature of ET-1, enable acute changes in ET-1 concentrations. This phenomenon undoubtedly facilitates the mediatory role of ET-1 in functional regression.

Conditions established during functional regression, that is endothelial cell activation and cytokine secretion, form the initial phase of structural regression, which assigns a key role to endothelium and macrophages in events leading to PCD in the corpus luteum (Fig. 8). $PGF_{2\alpha}$ sensitized endothelial cells might initiate a cascade of events leading to the recruitment and

transmigration of monocytes to the corpus luteum (MCP-1 may be the inducer of migration; Fig. 8). Migration and concomitant maturation to macrophages are followed by their activation, which could lead to PCD via secretion of TNFα. Whether endothelial cells are a primary target for PCD remains to be elucidated. We favour this notion, for regression of corpus luteum vasculature might cause local anoxia, which is an inducer of epithelial cell death.

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