Luteal Prostaglandin $F_{2\alpha}$: New Concepts of Prostaglandin $F_{2\alpha}$ Secretion and Its Actions within the Bovine Corpus Luteum[a] - Review -

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ABSTRACT : The corpus luteum (CL) is a temporary endocrine gland whose main function is to secrete progesterone to support pregnancy. On the other hand, the cyclic bovine CL has also been shown to be a site of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) production. Although there is general agreement that endometrial $PGF_{2\alpha}$ is an essential luteolysin in cattle, luteal $PGF_{2\alpha}$ seems to play a luteotropic role as an autocrine and/or paracrine factor, especially for the development and maintenance of the CL. This supposition is based on evidence that some of the prerequisites for autocrine/paracrine mechanisms are present, including local production of $PGF_{2\alpha}$ and the existence of specific binding sites within the CL. The purpose of this paper is to review the regulation of luteal $PGF_{2\alpha}$ secretion, its action on CL as an autocrine and/or paracrine factor and the receptivity of bovine CL to $PGF_{2\alpha}$. *(Asian-Aus. J. Anim. Sci. 2000. Vol. 13, No. 3 : 390-400)*

Key Words : $PGF_{2\alpha}$ Receptor, $PGF_{2\alpha}$, Corpus Luteum, Luteolysis, Cow

REGULATION OF PROSTAGLANDIN (PG)$F_{2\alpha}$ SECRETION IN BOVINE CL

Biosynthesis of $PGF_{2\alpha}$ in CL

Biosynthesis of the PGs in bovine corpus luteum (CL) is carried out in a stepwise fashion by two types of membrane-bound enzymes, cyclooxygenase and endoperoxide isomerases, and by the soluble enzyme peroxidase (Hansel and Dowd, 1986; Tsai and Wiltbank, 1997). Phospholipids are released from their esterified form by phospholipase (PL) A to a form of PG precursor, arachidonic acid (figure 1). The bovine CL contains relatively large amounts of arachidonic acid (approximately 3 mg/g tissue) and has the ability to metabolize this into a variety of products (Lukaszewska and Hansel, 1980). The first committing step of PG production is catalyzed by the enzyme cyclooxygenase (prostaglandin G/H synthase; PGHS) which converts arachidonic acid to the unstable form PGG_2. PGG_2 is quickly converted by peroxidase to PGH_2. PGG_2 and PGH_2 are unstable, biologically active molecules, called endoperoxides. Endoperoxide isomerase and other peroxidases convert PGH_2 to

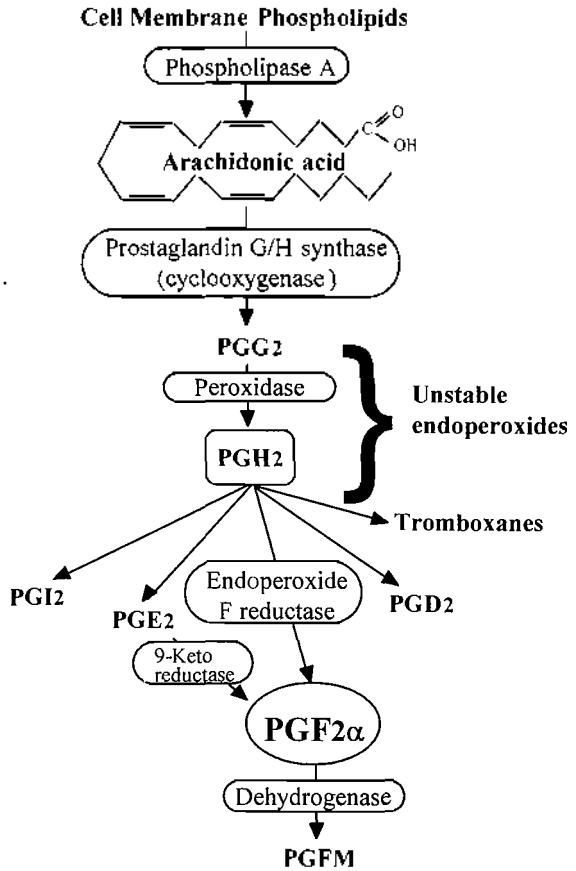

Figure 1. Pathway for prostaglandin (PG) $F_{2\alpha}$ biosynthesis in a luteal cell. See text for detailed description.

$PGF_{2\alpha}$, PGE_2, PGD_2, PGI_2 (prostacyclin) and to a 17-carbon cleavage product (e.g., tromboxanes; 12(s)-

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hydroxyheptadecatri-enoic acid-HHT) (Granström, 1981). PGs are not stored in tissues but are released immediately after synthesis (Bito, 1975). In many species the bioactive prostaglandins are formed very rapidly from their corresponding precursor, but then they are quickly converted to metabolites having much weaker activity. In ewe, $PGF_{2\alpha}$ in the bloodstream has a half-life of less than 1 min, since, in a single passage through lung, 99% of it is rapidly metabolised by 15-hydroxydehydrogenase and prostaglandin-13-keto-reductase to the stable metabolite 13,14-dihydro-15-keto-$PGF_{2\alpha}$ (Oates et al., 1980; Hansel and Dowd, 1986). Based on these observations, ovine luteal $PGF_{2\alpha}$ may play a role not systemically but locally within the CL. However, in cow, substantial amounts of $PGF_{2\alpha}$ pass through the lung without metabolic breakdown (Hansel and Dowd, 1986). Davis et al. (1984) found that about 35% of $PGF_{2\alpha}$ survived a first passage through the lungs and approximately 16% survived three circulations. Thus, luteal $PGF_{2\alpha}$ may also play a systemic role in the cow.

Influence of intraovarian factors on $PGF_{2\alpha}$ synthesis and output from CL

The bovine CL in the oestrous cycle produces $PGF_{2\alpha}$, with the highest concentration during the early luteal phase, and with decreased concentrations during the mid- and late luteal phase (Milvae and Hansel, 1983; Milvae et al., 1996; Rodgers et al., 1988). Ovarian oestradiol (E2), oxytocin (OT), and progesterone (P4) seem to be the physiological regulators of the synthesis and secretion of luteal $PGF_{2\alpha}$ during the oestrous cycle. In fact, E2 has been shown to be a potent stimulator of $PGF_{2\alpha}$ secretion in mid-cycle and late bovine CL (Grazul et al., 1988). Furthermore, we recently demonstrated that P4 and OT have an effect on the functionality of the bovine early CL in an autocrine and/or paracrine fashion (Skarzynski and Okuda, 1999). In order to remove the influence of OT and P4, which are produced by bovine luteal cells during culture, we added highly specific OT and P4 antagonists to the cultured luteal cells. In the early luteal cells, $PGF_{2\alpha}$ secretion was greatly reduced by atosiban (an OT antagonist) as well as by onapristone (a P4 antagonist). However, the P4 antagonist stimulated $PGF_{2\alpha}$ secretion in mid-cycle luteal cells. Therefore, it appears that P4 and OT cause the synthesis of $PGF_{2\alpha}$ in the early CL, although P4 inhibits $PGF_{2\alpha}$ synthesis in mid-cycle CL (figure 2). Pate (1988) also reported that P4 inhibited $PGF_{2\alpha}$ secretion in mature CL, but not in late CL. These findings indicate that ovarian steroids as well as OT affect the $PGF_{2\alpha}$ secretion from bovine CL in a stage-dependent fashion (table 1).

Although luteal OT action on luteal function including $PGF_{2\alpha}$ secretion (Grazul et al., 1989) is mediated by OT receptors present on luteal cells (Okuda et al., 1992; Okuda and Uenoyama, 1997), the mechanisms of ovarian steriod hormone action in CL have not been well studied. The primary mechanism of E2 and P4 involves regulation of gene transcription (Rories and Spelsberg, 1989). Therefore, E2 and P4 may act on CL by modulating PGHS mRNA expression and/or regulation of PGHS activity, as has been suggested to occur in the uterus (Salamonsen and Findlay, 1990; Poyser, 1995). Moreover, P4 has been known to enhance the activity of PGE_2-9-keto-reductase, which is responsible for conversion of PGE_2 into $PGF_{2\alpha}$ in the preovulatory follicles in sheep (Murdoch and Farris, 1988).

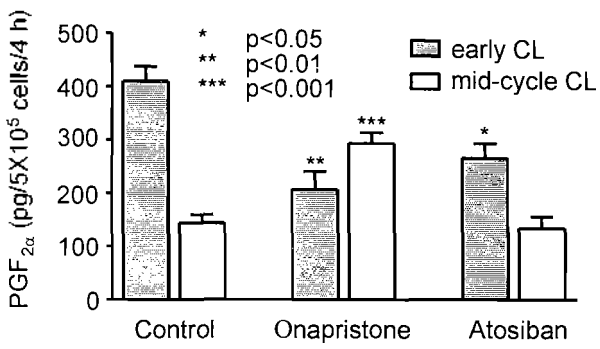

Figure 2. Effects of a highly selective P4 antagonist (onapristone; 100 μM) and a highly selective OT antagonist (atosiban; 1 μM) on prostaglandin (PG) $F_{2\alpha}$ secretion by bovine luteal cells dispersed from early CL (Days 4-5 of the oestrus cycle) and mid-cycle CL (Days 8-12). Adapted from Skarzynski and Okuda (1999), with permission.

Table 1. Simplified summary of the effects of intra-ovarian regulators on $PGF_{2\alpha}$ secretion from bovine luteal cells during the oestrous cycle. See text for details and references

Intraluteal regulators	Luteal phase		
	Early	Mid-cycle	Late
Oestradiol-17β	No effect	↑ ↑	↑
Progesterone	↑ ↑ ↑	↓ ↓	No effect
Oxytocin	↑ ↑ ↑	↑ ↑	↑
Noradrenaline	↑ ↑ ↑	↑ ↑	Not determined
Nitric oxide	No effect	↑ ↑	↑ ↑ ↑
Endothelin-1	↑ ↑ ↑	↑ ↑	↑
TNF_{α}	↑ ↑ ↑	↑ ↑	↑

$PGF_{2\alpha}$ secretion in bovine CL is regulated not only by products of luteal cells. Other potent intravarian factors such as cytokines, interleukin-1β (Townson and Pate, 1994; Del Vecchio and

Sutherland, 1997) and tumor necrosis factor-α (Benyo and Pate, 1992) also stimulate the secretion of $PGF_{2\alpha}$ from bovine luteal cells. In addition to cytokines, two products of other accessory cells (endothelial cells), endothelin-1 and nitric oxide, seem to also be crucial for the secretory function of CL. Moreover, the bovine ovary may synthesize adrenergic agents such as noradrenaline (Battista et al., 1989; Denning-Kendal et al., 1991). The neurotransmitters including noradrenaline and nitric oxide might also reach the CL via ovarian nerves and/or blood vessels (Majewski et al., 1995). All these factors may interact with each other and influence the secretory function of bovine CL including $PGF_{2\alpha}$ synthesis. Recently we showed that noradrenaline, nitric oxide and endothelin-1 (unpublished observation) may differently regulate $PGF_{2\alpha}$ secretion in cultured bovine luteal cells during the luteal phase (figure 3, table 1). Thus, intraovarian factors (E2, P4, OT, cytokines, neurotransmitters, and the products of endothelial cells) are involved in the regulation of luteal $PGF_{2\alpha}$ production in a stage-dependent manner (table 1). The stimulatory effect of these factors on $PGF_{2\alpha}$ secretion is maximal at the early luteal phase but relatively low towards the late luteal phase, suggesting that luteal $PGF_{2\alpha}$ may play one or more roles during development of bovine CL in an autocrine and/or paracrine fashion.

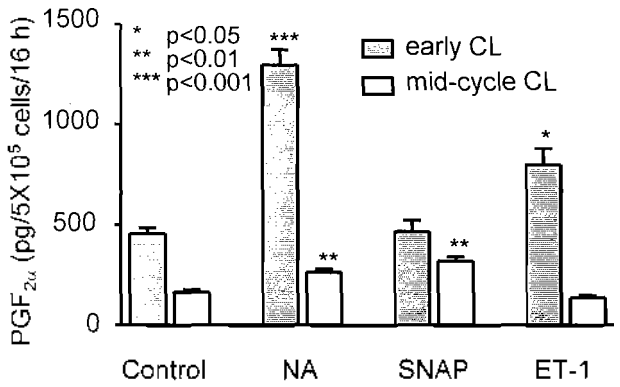

Figure 3. The effects of noradrenaline (NA; 10 μM), a nitric oxide donor (SNAP; 100 μM) and endothelin 1 (ET-1; 0.1 μM) on prostaglandin (PG) $F_{2\alpha}$ secretion by bovine luteal cells dispersed from early CL (Days 4-5 of the oestrous cycle) and mid-cycle CL (Days 8-12).

REGULATION OF THE FUNCTIONALITY OF $PGF_{2\alpha}$ RECEPTORS IN THE BOVINE CL

Number and affinities of $PGF_{2\alpha}$ receptors in the CL during different stages of the oestrus cycle

$PGF_{2\alpha}$ receptors in bovine CL have been identified by ligand binding techniques (Rao et al., 1979; Orlicky, 1990), by quantitative light microscope autoradiograph (Chegini et al., 1991) and recently by expression of specific mRNA (Sakamoto et al., 1995; Wiltbank et al., 1995; Mamluk et al., 1998). Although, previously it was suggested that both high and low affinity binding sites for $PGF_{2\alpha}$ occur in bovine CL (Rao, 1975; Orlicky, 1990), more recent studies indicated that there is only one population of high-affinity $PGF_{2\alpha}$ receptors (Sakamoto et al., 1994, 1995; Wiltbank et al., 1995). Sakamoto et al. (1995) used *in situ* hybridization to demonstrate that $PGF_{2\alpha}$ receptor mRNA was mostly located on large luteal cells (LLC). However, Mamluk et al. (1998) showed that expression of the $PGF_{2\alpha}$ receptor was only 3-fold higher in granulosa-derived luteal cells than in theca-derived luteal cells, indicating that $PGF_{2\alpha}$ receptor also may be present on the small luteal cells (SLC). In addition, they suggest that the non-steroidogenic cells in bovine CL such as endothelial cells may express $PGF_{2\alpha}$ receptors. These recent findings support the previous finding of Chegini et al. (1991) that both large and small steroidogenic luteal cells as well as endothelial cells contain specific $PGF_{2\alpha}$ binding sites. On the other hand, it has been demonstrated that the response of SLC to $PGF_{2\alpha}$ is higher than that of LLC (Alila et al., 1988; Alila et al., 1990a). Therefore, it seems likely that $PGF_{2\alpha}$ receptor mRNA and protein are not coordinatly expressed in bovine SLC. These findings suggest that the action of $PGF_{2\alpha}$ on luteal function is more complex than previously envisioned (Wiltbank et al., 1991) and involves both SLC and LLC, and in some cases, non-luteal cells.

$PGF_{2\alpha}$ receptors in bovine CL were also detected with an anti-sense riboprobe prepared from BC2211 cDNA (Sakamoto et al., 1994). The hybridization signal was strictly localized in CL obtained from the mid- and late luteal phases, but only a faint signal was detected in early CL. These findings support the finding of Rao et al. (1979) that the total binding of $PGF_{2\alpha}$ was low in the early luteal phase, increased in the mid-luteal phase and reached the highest value at the time of luteolysis.

Although $PGF_{2\alpha}$ binding in the late CL increased only 1.6-fold compard to the mid-cycle CL, the affinity in the late luteal phase was 203 fold higher than that of the mid-cycle CL (Rao et al., 1979). On the other hand, it has been recently reported that the affinity and number of $PGF_{2\alpha}$ binding sites progressively increased concomitantly with the expression of $PGF_{2\alpha}$ receptor mRNA (Northern blot analysis) from the early to the late luteal phase of the oestrous cycle (Sakamoto et al., 1995). Morever, high-affinity binding sites of $PGF_{2\alpha}$ were detected on the luteal membranes from the early CL. This is in agreement with a report that the concentration and affinity of $PGF_{2\alpha}$ receptor

were not different between early (days 2-4) and active (days 6-10) bovine CL (Wiltbank et al., 1995). These findings sugggest that $PGF_{2\alpha}$ is involved in regulation of the bovine CL during the entire luteal phase.

Intracellular signaling via $PGF_{2\alpha}$ receptor

The $PGF_{2\alpha}$ receptor in bovine CL was cloned and identified as a member of the 7-serpentine G protein-coupled receptor family (Sakamoto et al., 1994). Previous studies have indicated that the effects of $PGF_{2\alpha}$ in bovine CL appear to be mediated through the protein kinase C (PKC) second messenger system (Davis et al., 1987; Orwig et al., 1994). $PGF_{2\alpha}$ activates phospholiphase C, which causes hydrolysis of membrane phosphatidylinositol (PIP2) to inositol trisphosphate (IP3) and diacylglycerol (DAG). IP3 stimulates the release of calcium from intracellular stores, while DAG enhances the affinity of PKC for calcium, resulting in an increase of free intracellular calcium (Ca^{2+}) concentration and activation of PKC (Alila et al., 1990a; Davis et al., 1987). In contrast to the early signaling events, the downstream intracellular signaling events that can lead to transcriptional activation in response to $PGF_{2\alpha}$, are poorly understood. Recent data of Chen et al. (1998) have demonstred that $PGF_{2\alpha}$ activates the Raf/MEK1/ mitogen-activated protein kinase signaling cascade in bovine luteal cells. Moreover, the actions of $PGF_{2\alpha}$ were mimicked by a PKC activator (PMA). Therefore, the activation of Raf/MEK1/mitogen-activated protein kinase by $PGF_{2\alpha}$ may provide a mechanism to transduce signals initiated by $PGF_{2\alpha}$ receptors on the cell surface into the nucleus and may be associated with transcriptional activation of luteal genes (Davis et al., 1996; Chen et al., 1998).

The phosphorylation events associated with the activation of PKC and the IP3-mediated sustained elevations of Ca^{2+} are believed to regulate OT secretion (Orwig et al., 1994), to inhibit P4 and cause a cytotoxic effect (Wiltbank et al., 1991) as well as to stimulate P4 production (Alila et al., 1990b; Okuda et al., 1998). So, it should be emphasized that the varied actions of $PGF_{2\alpha}$ on the bovine CL upon binding to its G protein-coupled receptor are initiated by the same phospholipase C/DAG-IP3/Ca^{2+}-PKC pathway.

Desensitization of $PGF_{2\alpha}$ receptor responses

The newly formed bovine CL is resistant to treatment with a single injection of exogenous $PGF_{2\alpha}$ (Henricks et al., 1974; Beal et al., 1980). Moreover, the sensitivity of CL to $PGF_{2\alpha}$ seems to increase progressively toward the end of the luteal phase (Skarzynski et al., 1997). Since, as mentioned above, high-affinity binding sites of $PGF_{2\alpha}$ have been detected on the luteal membrane from early bovine CL (Sakamoto et al., 1995; Wiltbank et al., 1995), the unresponsiveness of early CL to $PGF_{2\alpha}$ is not a lack of $PGF_{2\alpha}$ receptors in the early bovine CL.

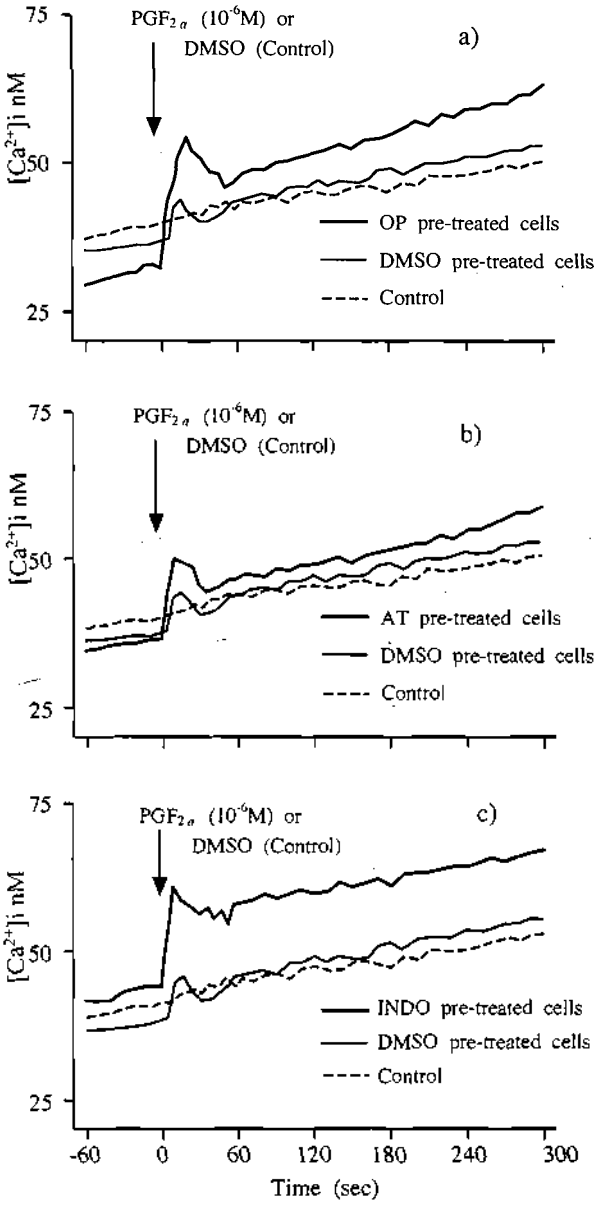

Figure 4. Effects of a progesterone antagonist (onapristone, OP) **(a)**, an oxytocin antagonist (atosiban, AT) **(b)** and an inhibitor of fatty acid cyclooxygenase (indomethacin, INDO) **(c)** on prostaglandin (PG) $F_{2\alpha}$-stimulated cytosolic free $Ca^{2+}[Ca^{2+}]_i$ in cells from early CL. Data are from one representative CL. Similar results were obtained in three other experiments. $PGF_{2\alpha}$ (1 μM) was added to Fura-2-loaded cells after 12 h of incubation with solvent (10% DMSO), OP (100 μM), AT (1 μm) or INDO (100 μM). Arrow indicate the time of $PGF_{2\alpha}$ or DMSO addition (control cells). Reprinted from Skarzynski and Okuda (1999), with permission.

Recently, we showed that the lack of response to $PGF_{2\alpha}$ in the early bovine CL and lower reaction of bovine CL to $PGF_{2\alpha}$ during the mid-luteal phase depend upon locally produced prostaglandins, OT and P4 (Skarzynski and Okuda, 1999). In order to measure the sensitivity of the CL to $PGF_{2\alpha}$ without the influence of intraluteal factors (P4, OT and $PGF_{2\alpha}$), we pre-exposed bovine luteal cells from early CL to a P4 antagonist (figure 4a), an OT antagonist (figure 4b) and an inhibitor of cyclooxygenase (figure 4c), and then stimulated the cells with $PGF_{2\alpha}$ (vertical arrows). Since $PGF_{2\alpha}$ rapidly increases intracellular free Ca^{2+} (Davis et al., 1987) in a dose- and threshold-dependent fashion, the intracellular level of free Ca^{2+} was used as an indicator of CL sensitivity to $PGF_{2\alpha}$. The effect of $PGF_{2\alpha}$ on the early CL was greater in cells receiving the three types of pretrement than in the controls (figure 4a, b and c, respectively). However, in the mid-cycle CL (data not shown), the effect of $PGF_{2\alpha}$ was magnified only by pre-exposure of the cells to an OT antagonist. However, in the mid-cycle CL, the effect of $PGF_{2\alpha}$ was magnified only by pre-exposure of the cells to an OT antagonist. These results indicate that luteal P4, OT and PGs are components of an autocrine/paracrine positive feedback cascade in bovine early to mid-cycle CL and may be responsible for the resistance of the early bovine CL to the action of exogenous $PGF_{2\alpha}$ (Skarzynski and Okuda, 1999).

The response of the CL to $PGF_{2\alpha}$ depends on the activation of membrane receptors and an intracellular signaling system in the luteal cells (Davis et al., 1987; Orwig et al., 1994). Therefore, the lack of response to $PGF_{2\alpha}$ in the early CL could be a consequence of receptor desensitization, that is, the general adaptive tendency of biological responses to wane over time. Desensitization of receptors is characterized by (1) a loss of receptor responsiveness to a stimulus of constant intensity (which includes the loss of the ability to regulate receptor number and affinity) and (2) an inability of the receptors to fully activate their second messenger systems. Generally, three separate processes may contribute to receptor desensitization: a functional uncoupling from the signaling effector system, mediated by phosphorylation of the receptor by distinct kinases; a direct inhibition of signal transduction through second-messengers, mediated by specific and non-specific proteins; and a sequesteration or internalization of the receptors away from the cell surface (Collins et al., 1991; Lohse, 1993; Davis et al., 1996; Wiltbank et al., 1992). Hormone-induced desensitization of G-protein-coupled receptors has been divided into two general categories, referred to as agonist-specific (or homologous) desensitization and agonist-nonspecific (or heterologous) desensitization (Lohse, 1993). Therefore, homologous desensitization of $PGF_{2\alpha}$ receptors in bovine CL might be due to stimulation by locally produced $PGF_{2\alpha}$, particularly in the early CL. Nevertheless, heterologous regulation by other ovarian factors can not be excluded (Skarzynski and Okuda, 1999). We have suggested that OT and P4, through their luteotropic action on early luteal to mid-luteal CL, may indirectly (via $PGF_{2\alpha}$; figure 2) or directly (via heterologous desensitization) affect the functionality of $PGF_{2\alpha}$ receptors and/or formation of second messengers. In support of this hypothesis, naturally occurring proteins that inhibit PKC activity have been detected in several tissues including the ovary (Melner, 1996). Recently, Juengel et al., (1998) reported that the increased resistance of the CL to $PGF_{2\alpha}$ during the early part of the oestrous cycle may be due to increased concentrations of mRNA encoding specific PKC inhibitors and the associated increase in the corresponding proteins in ovine CL. The inability of early CL to undergo regression following $PGF_{2\alpha}$ treatment may also be explained by downstream intracellular signaling events that can lead to different transcriptional activation of luteal genes in response to $PGF_{2\alpha}$ (Tsai and Wiltbank, 1998). Thus, it could be concluded that the different reactions of the CL to $PGF_{2\alpha}$ during the luteal phase and pregnancy can be explained not only by the changes in the concentration of $PGF_{2\alpha}$ receptors but also by changes in their sensitivity. Therefore, a number of different pathways (including receptor desensitization, changes in specific protein activity as well as gene expression) may account for the lack of $PGF_{2\alpha}$-induced luteolysis during the early luteal phase.

DIFFERENTIAL EFFECTS OF $PGF_{2\alpha}$ ON THE BOVINE CL

Luteotropic effects of $PGF_{2\alpha}$ during the development of the bovine CL

It is well known that $PGF_{2\alpha}$ secreted from the uterus mediates the functional and morphological regression of the bovine CL (Wiltbank et al., 1991; Poyser, 1995). However, the presence of $PGF_{2\alpha}$ in the bovine CL only roughly correlates with this luteolytic action, as $PGF_{2\alpha}$ concentrations in bovine CL are highest at the early luteal phase rather than at the mid- and late luteal phases. This discrepancy suggests that luteal $PGF_{2\alpha}$ may play roles that are different from those of endometrial $PGF_{2\alpha}$ in the bovine CL. The luteal $PGF_{2\alpha}$ could play a role in the development and maintenance of the bovine CL.

In spite of the existence of binding sites for $PGF_{2\alpha}$ in bovine CL during the entire luteal phase, $PGF_{2\alpha}$ causes a varity of responses with respect to P4 secretion *in vitro*, i.e. a luteolytic effect (Pate and Condon, 1989), a luteotropic effect (Hixon and Hansel, 1979) or no effect (Grazul et al., 1988). However, after the introduction of techniques for separating

small and large luteal cells (Alila et al., 1988; Meidan et al., 1992) and for preparing tissues with cell-to-cell contact (Miyamoto et al., 1993; Girsh et al., 1995), the effects of $PGF_{2\alpha}$ on bovine CL become relatively consistent. Surprisingly, $PGF_{2\alpha}$ did not prove to be luteolytic in these *in vitro* studies when added to either SLC or LLC preparations as well as during microdialysis of early and bovine mid-cycle CL. $PGF_{2\alpha}$ stimulates basal P4 secretion in bovine SLC (Alila et al., 1988). On the other hand, $PGF_{2\alpha}$ had no effect on basal P4 production by LLC; however, it inhibited LH- and lipoprotein-stimulated P4 production (Alila et al., 1988; Pate and Condon, 1989). Recently, we showed that $PGF_{2\alpha}$ plays a role as a luteotropic agent in an autocrine and/or paracrine manner in bovine luteal cells (a mixture of SLC and LLC; Okuda et al., 1998), and that the stimulatory action of $PGF_{2\alpha}$ on luteal steroidogenesis appears to be mediated by PKC (figure 5). Although treatment of the luteal cells with $PGF_{2\alpha}$ resulted in an increase of P4 production, the stimulatory effect of $PGF_{2\alpha}$ was no longer evident after down-regulation of PKC with a tumor-promoting ester, phorbol 12-myristate 13-acetate (PMA) (figure 5).

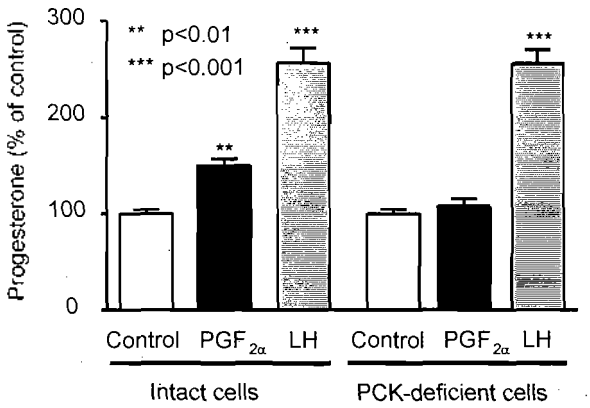

Figure 5. Effects of prostaglandin (PG) $F_{2\alpha}$ and LH on progesterone secretion by intact or protein kinase C (PKC)-deficient luteal cells. The cells (5×10^4 cells) were exposed to $PGF_{2\alpha}$ (1 μM) or LH (50 ng/ml) following 24 h preincubation with 1 μM phorbol 12-myristate 13-acetate. The concentrations of progesterone in controls of intact and PKC-deficient cells were approximately 335 and 300 ng/ml, respectively. Reprinted from Okuda et al. (1998), with permission.

This indicates that the stimulatory effect of $PGF_{2\alpha}$ on the P4 secretory function of bovine CL is mediated by the activation of PKC. These data agree with the findings of Alila et al. (1990a, b) concerning the effect of $PGF_{2\alpha}$ on the second messenger system in small bovine luteal cells. However, luteal $PGF_{2\alpha}$ seems to be luteotropic at the early and mid-luteal phases, but no longer luteotropic at the late luteal phase despite the relatively high local production (Miyamoto et al., 1993).

In addition to its direct stimulatiry effect on P4 secretion, $PGF_{2\alpha}$ may affect P4 secretion indirectly by regulating the secretion as well as the action of other luteotropic factors in the CL. Luteal OT was found to be a potent stimulator of P4 output and production from bovine CL (Miyamoto and Schams, 1991; Sakumoto et al., 1996). This effect of OT was more evident during the early luteal phase and decreased during the mid to late luteal phase (Miyamoto and Schams, 1991). The fact that the change in the effect of OT parallels the change in the concentrations of OT receptors on bovine luteal cells (Okuda et al., 1992) demonstrates the physiological relevance of luteal OT as a luteotropic autocrine and/or paracrine factor, especially during CL development. Therefore, the stimulatory effect of $PGF_{2\alpha}$ on luteal OT secretion (Abdelgadir et al., 1988; Skarzynski and Okuda, 1999) may be a component of an autocrine/paracrine positive feedback cascade in bovine early to mid-cycle CL. Moreover, luteal $PGF_{2\alpha}$ seems to regulate the functionality of OT receptors in the bovine ovary (Okuda and Uenoyama, 1998). Our previous study (Okuda et al., 1995) indicated that $PGF_{2\alpha}$ could increase the concentration of OT receptors in cultured bovine luteal cells (figure 6).

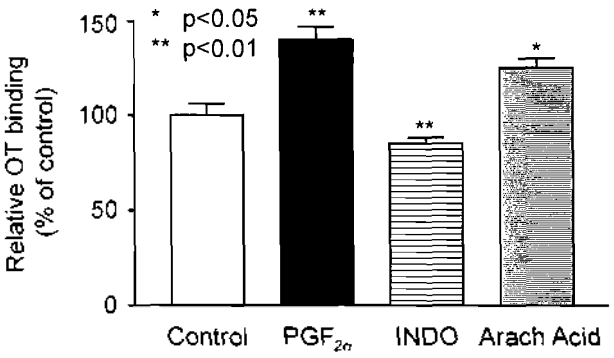

Figure 6. Effects of prostaglandin (PG) $F_{2\alpha}$ (0.1 μM), indomethacin (INDO; 28 μM) and arachidonic acid (Arach Acid; 100 μM) on the specific binding for oxytocin in cultured bovine luteal cells from mid-cycle CL (Days 8-12 of the oestrous cycle). The cells were incubated with regents for 15 h ($PGF_{2\alpha}$) or 28 h (INDO and Arach Acid). The specific binding of controls was approximately 7.4% (n=18) using 5×10^5 cells per well. Adapted from Okuda et al. (1995), with permission.

Furthermore, significant reduction in the specific binding of OT occurred following the inhibition of luteal PG synthesis by indomethacin, which is known to be an inhibitor of PGG/H synthase. On the other

hand, since arachidonic acid, a precursor of PGs, caused concomitant increases in $PGF_{2\alpha}$ production and specific binding of OT, it is possible that the subsequent increases in the concentration of $PGF_{2\alpha}$ induced the binding of OT. Moreover, in this study, the specific binding of OT in the PKC-deficient luteal cells was not affected by $PGF_{2\alpha}$ stimulation. All these findings suggest that $PGF_{2\alpha}$ may be one of the potent regulators of luteal OT receptors in an autocrine and/or paracrine manner, and that its action is mediated by PKC. Considering all these facts, it should be emphasized that locally produced $PGF_{2\alpha}$ may play different effects within the bovine CL including direct and/or indirect luteotropic effects. Stimulating and inhibiting effects may be regulated by autocrine/paracrine mechanisms and may depend on the stage of the luteal phase (Miyamoto et al., 1993; Skarzynski and Okuda, 1999).

Supporting roles of luteal $PGF_{2\alpha}$ in the regression of the bovine CL at the end of the luteal phases

The praticipation of intraluteal $PGF_{2\alpha}$ in luteolysis is suggested by several types of indirect evidence in different species including the cow (Neill et al., 1969; Beling et al., 1970; Beal et al., 1980; Lytton and Poyser, 1982). It has been suggested that a functional $PGF_{2\alpha}$ autoamplification system is essential for the completion of luteolysis in the CL (Beal et al., 1980; Schramm et al., 1983; Zarco et al., 1988). Tsai and Wiltbank (1997) reproted that $PGF_{2\alpha}$, acting through the PKC/free Ca^{2+} pathway, can stimulate luteal cells to express PGG/H synthase and produce $PGF_{2\alpha}$ in ovine CL. This luteal $PGF_{2\alpha}$ is likely to have an autocrine and/or paracrine function to augment the luteolytic effect of $PGF_{2\alpha}$ of uterine origin. Interestingly, this autoamplification cascade, as evidenced by PGG/H synthase expression and $PGF_{2\alpha}$ production, is not induced by $PGF_{2\alpha}$ in the early bovine CL (Tsai and Wiltbank, 1998). Since LLC contain a higher number of $PGF_{2\alpha}$ receptors and are more sensitive to luteolytic effects of $PGF_{2\alpha}$ than are SLC (Heath et al., 1983; Alila et al., 1988), one could assume that the LLC are targets of the luteolytic effects of $PGF_{2\alpha}$. However, which mechanisms within LLC are involved in the luteolytic effect of $PGF_{2\alpha}$ is not well understood.

At first, an alteration in the number of receptors for LH and an uncoupling of adenylate cyclase from the LH receptor was suggested as the mechanism of $PGF_{2\alpha}$-induced luteolysis (Spicer et al., 1981). However, the decrease in LH-receptors was found to occur at least 12 h after the decrease in P4 level (Pate, 1994). Thus the loss of gonadotirpin receptors may be a chronic effect of $PGF_{2\alpha}$ rather than the initial cause of luteolysis. Limiting the substrate supply for steroidogenesis could be another way by which $PGF_{2\alpha}$ evokes luteolysis. However, $PGF_{2\alpha}$ has no effect on the uptake of low-density or high-density lipoprotein in bovine luteal cells (Pate and Condon, 1989; Grusenmeyer and Pate, 1992). Alternatively, $PGF_{2\alpha}$ may act by inhibiting phospholipid synthesis, which is important for stimulation of cholesterol side-chain cleavage, or by inhibiting the transfer of cholesterol from the outer to the inner mitochondrial membrane (Strauss et al., 1982). $PGF_{2\alpha}$ may also suppress *de novo* sterol synthesis, further limiting the pool of cholesterol that would be available for P4 synthesis (Pate and Condon, 1989). Finally, the appearance of endonuclease activity, as evidenced by the formation of oligonucleosomes, suggests that apoptosis occurs during luteal regression in cattle (Juengel et al., 1993; Pate and Townson, 1994). This may be one of the ways by which $PGF_{2\alpha}$ induces luteolysis. However, little is known about the intracellular mechanisms in luteal cells that can directly lead to inhibiting P4 secretion in response to $PGF_{2\alpha}$.

The acute decrease in plasma P4 level during the initial stage of luteolysis are correlated with reduced levels of mRNA for 3β-hydroxysteroid dehydrogenase (3β-HSD), but not for cytochrome P450 side-chain cleavage (Tian et al., 1994). Although $PGF_{2\alpha}$ decreases mRNA for 3β-HSD, the level of the protein does not decrease in parallel with decrease of its mRNA (Rodgers et al., 1995). In contrast, acute changes in steroidogenesis appear to be associated with changes in active steroidogenic acute regulatory protein (StAR) without any changes at the gene level (Tian et al., 1994; Stocco and Clark, 1996; Tsai and Wiltbank, 1998). Although it seems that the molecular mechanisms that mediate the decreased production of P4 during luteolysis involve the down-regulation of genes encoding steroidogenic enzymes, the mechanisms by which $PGF_{2\alpha}$ directly effects luteolysis in bovine CL have not been completely clarified.

The influence of cell-to-cell communications on $PGF_{2\alpha}$ actions within bovine CL

Although $PGF_{2\alpha}$ has a cytotoxic effect on bovine CL *in vivo*, experiments using long-term culture of bovine luteal cells have demonstrated that $PGF_{2\alpha}$ is not capable of directly inducing cytolysis (Fairchild and Pate, 1987; Girsh et al., 1995). The bovine CL is composed of several cell types, including small and large steroidogenic cells, epithelial cells, connective tissue cells, and others such as immune cells (Lei et al., 1991; Fields and Fields, 1996). It has been clearly demonstrated that the responses of cultured luteal cells to $PGF_{2\alpha}$ are different among LLC, SLC and a mixture of luteal and non-steroidogenic cells (Alila et al., 1988, 1990b; Hansel et al., 1991; Girsh et al., 1995). Therefore, it appears that cell-to-cell contact,

and communication between luteal cells and non-luteal cells, and within luteal cells are essential for luteogenesis and CL development (Jablonka-Shariff et al., 1993), and for the maintenance (Redmar et al., 1991; Del Vecchio et al., 1995) and regression of bovine CL (Girsh et al., 1996; Pate, 1996). It has also been suggested that $PGF_{2\alpha}$-induced inhibition of P4 production in luteal cells is not due to a specific cytotoxic effect (Girsh et al., 1995). In support of this hypothesis, the inhibitory effect of $PGF_{2\alpha}$ is evident only in the presence of cAMP-elevating agents (Alila et al., 1988; Girsh et al., 1995) and in the presence of both steridogenic luteal cells and endothelial cells (Girsh et al., 1995, 1996). In addition to the endothelial cells, the immune cells (i.e., macrophages, cytotoxic T cells and antibody-producing B cells) may be involved in regulation of the bovine CL functions including $PGF_{2\alpha}$ production and its actions (Pate, 1996). As evidence of the direct cell-to-cell interactions, immune cells and luteal cells likely communicate via secreted products i.e., cytokines (Pate, 1995, 1996). All these factors may act on CL either independently or in concert to modify the actions of $PGF_{2\alpha}$. Moreover, luteal $PGF_{2\alpha}$ can also modulate the activity of immune cells and may regulate the immune processes within the bovine CL (Benyo et al., 1991; Pate, 1994). Together, these findings raise the possibility that the non-steroidogenic cells of bovine CL (endothelial and immune cells) have a role in the steroidogenic functions of the luteal cells. Consequently, the cellular interactions within the bovine CL may be physiologically relevant to the luteotropic and luteolytic processes in this gland.

CONCLUDING REMARKS

The CL of the oestrous cycle in the cow is a dynamic endocrine organ with a functional life span of approximately 15-17 days. Adequate luteal function is crucial for determining the physiological duration of the oestrous cycle and for achieving a successful pregnancy. The bovine CL grows very fast and regresses within two days. Therefore, luteal development and luteolysis are both essential processes that regulate the duration and function of the CL. Generally, there is agreement that $PGF_{2\alpha}$ is a luteolysin in cattle. However, several observations indicate that luteal $PGF_{2\alpha}$ might play a luteotropic role within the CL, especially in the development and maintenance of the CL. This supposition is based on the presence of some of the prerequisites for autocrine/paracrine mechanisms, including local production of $PGF_{2\alpha}$ and their specific binding sites within the CL. These autocrine/paracrine mechanisms affect the changes in productivity, receptivity and activity of luteal as well as non-luteal cells during the luteal phase. Several intraovarian factors such as OT, P4, tumor necrosis factor-α, endothelin-1 and noradrenaline were found to be potent stimulators of $PGF_{2\alpha}$ secretion by the bovine

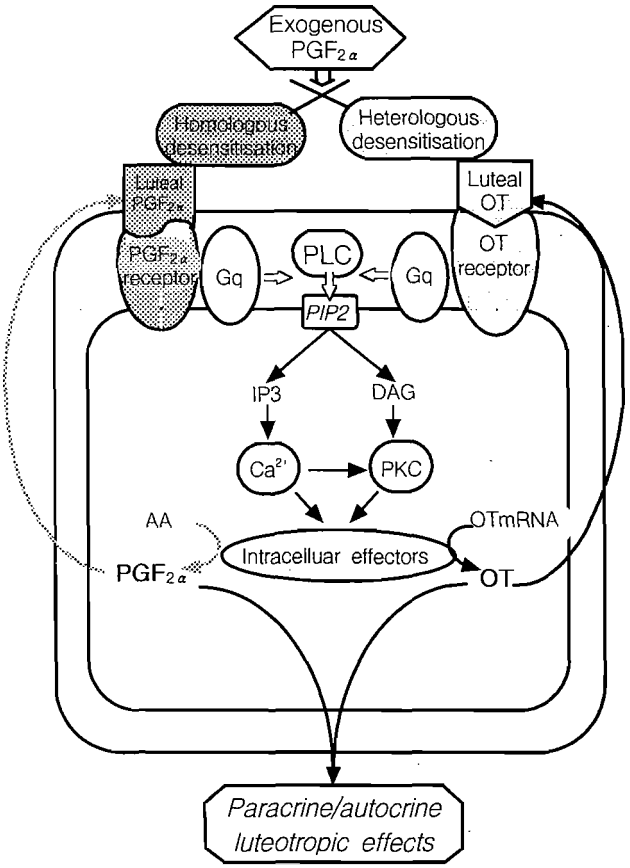

Figure 7. Conceptual model showing a positive autocrine/paracrine feedback loop between prostaglandin (PG) $F_{2\alpha}$ and oxytocin (OT) in a luteal cell during the early luteal phase. Luteal $PGF_{2\alpha}$ via G-protein (Gq) activates phospholipase C (PLC), which causes hydrolysis of membrane phosphatidylinositol (PIP2) to inositol triphosphate (IP3) and diacylglycerol (DAG). IP3 stimulates the release of intracellular Ca^{2+}, while DAG activates protein kinases C (PKC). Both, Ca^{2+} and PKC activate intracellular effectors, which evoke physiological responses of the cell including stimulation of OT secretion. $PGF_{2\alpha}$ may also regulate OT receptor functionality. In the reverse direction, OT activating the PKC-Ca^{2+} second messenger pathway stimulates $PGF_{2\alpha}$ secretion. Both hormones, which affect luteal cells as autocrine/paracrine luteotropic factors, may desensitize $PGF_{2\alpha}$ receptors and cause early bovine CL to become unresponsive to exogenous $PGF_{2\alpha}$. Homologous desensitization of $PGF_{2\alpha}$ receptors is possible during long-lasting stimulation by locally produced $PGF_{2\alpha}$. Apparently, OT as well as P4 can also modulate the $PGF_{2\alpha}$ signaling pathway through heterologous desensitization.

CL. The stimulation of $PGF_{2\alpha}$ secretion is maximal at the early luteal phase and decreases toward the late luteal phase, supporting the view that luteal $PGF_{2\alpha}$ may play autocrine/paracrine roles during luteal development. However, the sensitivity of bovine CL to $PGF_{2\alpha}$ increases progressively toward the end of the luteal phase and the newly formed bovine CL is resistant to treatment with exogenous $PGF_{2\alpha}$. This unresponsiveness of the early CL to $PGF_{2\alpha}$ is not due to a lack of high-affinity $PGF_{2\alpha}$ receptors. Our recent results indicate that luteal PGs, P4 and OT are components of an autocrine/paracrine positive feedback cascade in bovine CL and are responsible for the resistance of the early bovine CL to the action of exogenous $PGF_{2\alpha}$ (figure 7). The lack of response to $PGF_{2\alpha}$ in early bovine CL is regulated at the receptor level and/or the post-receptor level (figure 7). Multiple processes seem to be involved in regulating the responsiveness of the $PGF_{2\alpha}$ receptor-coulped protein G systems. One of these processes is the homologous desensitization of $PGF_{2\alpha}$ receptors in the CL, which may be due to long-lasting stimulation by $PGF_{2\alpha}$ produced in the ovary. Moreover, OT and P4 through their luteotropic actions on the early to mid-CL, may indirectly (via $PGF_{2\alpha}$) or directly (via heterologous desensitization) affect the functionality of $PGF_{2\alpha}$ receptors and/or formation of second messengers. The reverse effects, in which $PGF_{2\alpha}$ may regulate OT secretion as well OT receptor functionality may also occur. Moreover, luteal $PGF_{2\alpha}$ seems to be a luteotropic factor during the formation and development of the CL and may directly stimulate P4 as well OT secretion. This positive feedback cascade may decrease the sensitivity of CL to exogenous $PGF_{2\alpha}$ action that could be a mechanism for protection against premature luteolysis during luteal development. Nevertheless, at the end of the luteal phase, $PGF_{2\alpha}$ and OT may interact and activate luteal and non-luteal cells to initiate functional and morphological luteolysis.

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