Molecular regulation of luteal progesterone synthesis in domestic ruminants

J. L. Juengel and G. D. Niswender

Animal Reproduction and Biotechnology Laboratory, Colorado State University, Fort Collins, CO 80523-1683, USA

Regulation of progesterone secretion from the corpus luteum during the oestrous cycle requires the integration of multiple signals to achieve the appropriate amount of progesterone to maximize reproductive efficiency. Development of a mature corpus luteum capable of secreting sufficient amounts of progesterone is dependent upon the pituitary hormones LH and growth hormone (GH). Continued secretion of progesterone from the mature corpus luteum is also dependent upon pituitary hormones. If pregnancy does not occur, prostaglandin F2α (PGF2α) of uterine origin causes a precipitous decrease in progesterone secretion and demise of the corpus luteum. A major point of regulation of progesterone secretion by both luteotrophic and luteolytic hormones appears to be regulation of transport of cholesterol through the mitochondrial membranes to cytochrome P450scc. It is likely that both luteotrophic and luteolytic hormones regulate steroidogenic acute regulatory protein (StAR), which facilitates transport. Regulation may be occurring through increases or decreases in gene transcription, translation efficiency or post-translational modifications such as phosphorylation. Thus, although synthesis of progesterone is a complex process, both positive and negative regulation of the process appears to occur primarily at a single step (transport of cholesterol to the inner mitochondrial membrane) in the pathway.

Introduction

The corpus luteum, which secretes progesterone, is a transient endocrine gland formed from follicular cells following ovulation. Progesterone is necessary for maintenance of pregnancy in all domestic ruminants. Inadequate luteal secretion of progesterone is a major cause of early embryonic mortality (Nancarrow, 1994; Zavy, 1994). However, if fertilization does not occur, the corpus luteum must stop producing progesterone to allow the complex series of events that results in another ovulation.

In domestic ruminants, the primary luteotrophic hormones, which support the development and function of the corpus luteum, are LH and GH. In addition, locally produced prostaglandins (PG) of the E and I series, and insulin-like growth factors (IGF) probably support luteal function. The luteolytic hormone that causes decreased secretion of progesterone and demise of luteal cells is PGF2α. This review will focus on how luteotrophic and luteolytic hormones regulate progesterone synthesis at the cellular and molecular levels.

Luteal Steroidogenic Pathway

For a better understanding of the way in which these hormones regulate secretion of progesterone from the corpus luteum it is first important to understand how steroidogenic cells produce progesterone (Fig. 1). Progesterone is made from the precursor cholesterol. Under normal conditions in vivo, the majority of the cholesterol used for synthesis of all steroid hormones is obtained from high density lipoprotein (HDL) or low density lipoprotein (LDL) (Gwynne and Strauss, 1982). Once
cholesterol is taken into the cell, it can be shuttled into the steroid biosynthetic pathway or stored as cholesterol esters in the form of lipid droplets (Gwynne and Strauss, 1982). Cholesterol can be released from these stores by cholesterol esterase, when demand for cholesterol exceeds the supply. During steroidogenesis, cholesterol moves through the cytoplasm to the mitochondria, where the side chain is cleaved. This cytoplasmic translocation is dependent upon the cytoskeleton (Jefcoate et al., 1992) and probably involves sterol-binding proteins. Cholesterol must then pass through the outer to the inner mitochondrial membrane where the enzyme complex involved in cholesterol side-chain cleavage is located. Transport of cholesterol across the mitochondrial membranes appears to be the rate-limiting step of steroidogenesis (Stocco and Clark, 1996). It has been proposed that the
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recently identified steroidogenic acute regulatory (STAR) protein facilitates transport of cholesterol to cytochrome P450 side-chain cleavage enzyme (P450scc). The hypothesis that STAR is crucial to transport of cholesterol is supported by several lines of evidence. First, there is preliminary evidence that STAR is capable of binding cholesterol (Liu et al., 1996). Second, STAR has been detected in the inner mitochondrial membrane where cholesterol side-chain cleavage occurs (King et al., 1995). Third, naturally occurring mutations in the STAR gene severely limit steroidogenesis in adrenal and gonadal tissues (Lin et al., 1995). Finally, gonadal tissue collected from patients with mutated STAR protein is capable of producing normal amounts of steroids if provided with membrane permeable cholesterol analogues which by-pass the usual route of cholesterol transport (Miller, 1996).

Another protein that appears to facilitate transport of cholesterol to the side chain cleavage enzyme complex is the peripheral benzodiazepine receptor (PBR; Papadopoulos et al., 1997a). Targeted deletion of PBR resulted in loss of steroidogenic capacity of Leydig cells, which was restored when the cells were supplied with membrane permeable cholesterol analogues (Papadopoulos et al., 1997b). In addition, PBR appears to associate with a voltage-dependent anion channel (VDAC) and molecular modelling indicates that the PBR–VDAC complex will form a pore permeable to cholesterol which spans the mitochondrial membranes (Papadopoulos et al., 1997a). However, little information is available regarding regulation of this system in luteal tissue and thus, the role of this complex in control of luteal progesterone synthesis in domestic ruminants is not known. Once in the mitochondria, cholesterol is converted to pregnenolone by the side chain cleavage enzyme complex which comprises cytochrome P450scc, adrenodoxin and adrenodoxin reductase proteins. Pregnenolone is then converted to progesterone by 3β-hydroxysteroid dehydrogenase/Δ^5, Δ^4 isomerase (3β-HSD) in the smooth endoplasmic reticulum.

Steroidogenic Cells of the Corpus Luteum

The corpus luteum contains at least two types of steroidogenic cell that differ in morphological as well as biochemical characteristics. In mature ovine luteal tissue, small luteal cells are 8–22 μm in diameter, have lipid droplets, abundant smooth endoplasmic reticulum and are spindle shaped (Niswender et al., 1985). These cells have receptors for LH and respond to this hormone with a 6–12-fold increase in steroid production (Fitz et al., 1982; Alila et al., 1988a). Treatment of small luteal cells with PGI2 also increases progesterone synthesis (Fitz et al., 1984; Alila et al., 1988b). In the ewe, small luteal cells do not have high-affinity receptors for PGF2α, or PGE2 (Fitz et al., 1982; Chegini et al., 1991). In the cow, mRNA encoding PGF2α-receptor (Mamluk et al., 1998) as well as PGF2α and PGE2 (Chegini et al., 1991) binding sites have been detected in small luteal cells.

Large luteal cells are more spherical, greater than 22 μm in diameter, contain abundant quantities of smooth and rough endoplasmic reticulum, have protein containing secretory granules and in cows, but not sheep, contain lipid droplets (Niswender et al., 1985). Although large luteal cells contain receptors for LH (Harrison et al., 1987; Chegini et al., 1991), binding of LH to its receptor does not increase secretion of progesterone from these cells (Rodgers et al., 1983; Hoyer et al., 1984; Alila et al., 1988a). Large luteal cells also contain receptors for prostaglandins F2α, E2, and I2 (Fitz et al., 1982; Chegini et al., 1991) as well as GH (Lucy et al., 1993) and IGF-I (Perks et al., 1995). Basal secretion of progesterone is 10–40 fold higher in large than in small cells but treatment with stimulatory hormones, such as PGE2 or PGL, only increases secretion of progesterone from ovine large luteal cells 2–4 fold (Fitz et al., 1984). Prostaglandin F2α decreases secretion of progesterone from these cells (Wiltbank et al., 1991). It has been calculated that the majority of the progesterone (≤80%) secreted from the mature ovine corpus luteum is derived from large luteal cells (Niswender et al., 1985) although this may be controversial (Rodgers et al., 1983).

Regulation of Progesterone Secretion During the Oestrous Cycle

Changes in the concentration of progesterone in sera normally observed during the oestrous cycle are due to changes in luteal blood flow, size and number of steroidogenic cells and changes in the
Fig. 2. Changes in concentrations of progesterone in sera (serum P₄), luteal weight, blood flow to the ovary bearing a corpus luteum, and numbers and sizes of small (S) and large (L) luteal cells (LC) over the ovine oestrous cycle are presented in the top two panels. Changes in mRNA encoding proteins important for cholesterol uptake and transport to the mitochondria (high density lipoprotein-
steroidogenic capacity of luteal cells (Fig. 2). Increases in concentrations of progesterone in sera during the early luteal phase are paralleled by increases in blood flow (Niswender et al., 1976), luteal weight and numbers and sizes of steroidogenic luteal cells (Fig. 2; Farin et al., 1986). In contrast, mRNAs encoding many proteins important for steroidogenesis are already expressed at high concentrations by day 3 of the ovine oestrous cycle (Fig. 2; Juengel et al., 1994, 1995a; Tandeski et al., 1996). For this communication, data regarding mRNA encoding important proteins that regulate the functions of luteal cells are expressed, in all cases, as steady-state concentrations. Although the total amount of mRNA encoding most of these proteins in the corpus luteum would increase as the numbers and size of steroidogenic cells increase during luteal development, it is the concentrations of each species of mRNA versus other mRNAs which should control the activity of individual cells. Therefore, steady-state concentrations of mRNA should reflect the ability of an individual mRNA to influence cellular function. It does not appear that expression of any of these mRNAs severely limits secretion of progesterone during the early luteal phase.

Both mRNA and the numbers of receptors for LH (LH-R) increase as luteal development progresses (Fig. 2, Diekman et al., 1978a; Spicer et al., 1981; Garverick et al., 1985; Guy et al., 1995). Thus, it is possible that cellular growth and division and increased progesterone secretion are driven by increasing the responsiveness of individual cells to LH. Progesterone increases expression of LH-R in luteal cells from bovine corpora lutea collected during the early luteal phase (Jones et al., 1992). In addition, luteal concentrations of mRNA encoding IGF-I, IGF-II and the receptor for IGF-I increase during luteal development (Einspanier et al., 1990; Perks et al., 1995; Juengel et al., 1997b) providing additional potential mechanisms for hormonal support of normal luteal growth. During luteal regression, the decrease in concentrations of progesterone in serum is closely associated with reduced blood flow to the corpus luteum (Niswender et al., 1976) and decreased steroidogenic capacity of luteal cells (McGuire et al., 1994; Juengel et al., 1995a; Tandeski et al., 1996). The number of steroidogenic cells decreases later during luteal regression (Braden et al., 1988) and is not associated with the acute downregulation of progesterone synthesis seen during luteal regression. The focus of the remainder of this review will be regulation of steroidogenic capacity of luteal cells as well as their ability to respond to luteotrophic and luteolytic hormones.

Acquisition of maximum steroidogenic capability

The preovulatory LH surge causes release of the oocyte from the follicle and differentiation of follicular cells into luteal cells (luteinization). Luteinization is characterized by increased steroid production and a switch from producing oestradiol to progesterone. Not surprisingly concentrations of LDL-R, StAR, P450scc and 3β-HSD increase during this time (mRNA, protein, or both; Rodgers et al., 1986, 1987; Couet et al., 1990; Voss and Fortune, 1993a; Juengel et al., 1994; Pescador et al., 1996). In addition, enzymes important for oestrogen synthesis (aromatase and 17α-hydroxylase cytochrome P450) were greatly decreased in the newly formed bovine corpus luteum (Rodgers et al., 1986, 1987; Voss and Fortune, 1993b). Cells also become more responsive to LH (Diekman et al., 1978a) and GH (Lucy et al., 1993). Receptors for PGE2a also increase during luteinization to reach maximum numbers per large luteal cell shortly after ovulation (Wilbank et al., 1993). Thus, during luteinization, the newly formed corpus luteum gains increased ability to synthesize progesterone and respond to regulatory hormones.
The corpus luteum continues to secrete increasing amounts of progesterone for several days following luteinization, and continued development of the corpus luteum is dependent upon pituitary support (Farin et al., 1990; Juengel et al., 1995b). Supply of cholesterol probably does not limit secretion of progesterone from the corpus luteum during development, as steady-state concentrations of mRNA encoding HDL-binding protein (HDL-BP; Tandeski et al., 1996) and LDL-R (Rodgers et al., 1987; Tandeski et al., 1996) were maximal early in the oestrous cycle (Fig. 2), and hypophysectomy of ewes did not decrease expression of these mRNAs (Tandeski et al., 1996). Steady-state concentrations of mRNA encoding StAR or STAR protein also peak early in the oestrous cycle (Juengel et al., 1996a; Pescador et al., 1996). However, removal of pituitary support during luteal development severely reduces mRNA encoding StAR and replacement of LH or GH prevented this decrease (Juengel et al., 1995a). Thus, expression of normal amounts of mRNA encoding StAR, and presumably protein since these two variables are highly correlated (Pescador et al., 1996), appears essential for maximal secretion of progesterone. Conversion of cholesterol to pregnenolone by the P450scc enzyme complex may limit secretion of progesterone during development of the corpus luteum as mRNA, or protein, or both for P450scc and adrenodoxin increase during luteal development (Rodgers et al., 1986; 1987; Juengel et al., 1994). Removal of the pituitary gland prevents the normal increases in mRNA encoding P450scc observed during the ovine oestrous cycle, and replacement of LH or GH supports normal expression of this mRNA (Juengel et al., 1995b). Conversion of pregnenolone to progesterone by 3β-HSD does not appear to limit secretion of progesterone from the corpus luteum. Concentrations of mRNA encoding 3β-HSD, as well as 3β-HSD protein and enzyme activity reach maximum values early in the oestrous cycle and appear to be in great excess (Couet et al., 1990; Wiltbank et al., 1993; Juengel et al., 1994). In addition, maximal expression of 3β-HSD mRNA, which is dependent upon LH, was not required for maximal secretion of progesterone (Juengel et al., 1995b).

The increase in the number of receptors for LH observed during luteal development (Diekman et al., 1978a; Spicer et al., 1981, Garverick et al., 1985) was preceded by an increase in mRNA encoding this receptor (Guy et al., 1995). However, removal of the pituitary gland during luteal development did not prevent expression of normal concentrations of LH-R in luteal tissue (Farin et al., 1990; Juengel et al., 1995b). Thus, reduced expression of the LH-R was not limiting secretion of progesterone in hypophysectomized ewes. In contrast, while mRNA encoding the receptor for GH (GH-R) is expressed at maximal concentrations in corpora lutea by day 3 of the ovine oestrous cycle, removal of the pituitary gland caused a decrease in expression of this mRNA (Juengel et al., 1997b). Somewhat surprisingly, LH, but not GH, supported normal expression of GH-R mRNA (Juengel et al., 1997b). However, concentrations of progesterone in sera of GH-treated, hypophysectomized ewes were not different from those in pituitary-intact control ewes. Thus, secretion of progesterone in hypophysectomized ewes was not probably limited by a lack of GH binding. Similarly, although concentrations of mRNA encoding IGF-I increase during luteal development, this increase in expression of IGF-I mRNA was not necessary for normal progesterone biosynthesis (Juengel et al., 1997b). In contrast, increases in luteal weight were associated with increased expression of IGF-I mRNA (Juengel et al., 1997b). Thus, concentrations of progesterone in sera are not tightly linked to luteal expression of IGF-I mRNA, but increases in luteal weight were associated with increases in this mRNA.

Maintenance of maximal steroidogenic capacity

Once the corpus luteum is fully formed, it does not appear to require pulsatile LH release to maintain secretion of progesterone at normal, mid-luteal phase values in either sheep or cattle (McNeilly et al., 1992; Peters et al., 1995). However, basal amounts of LH are necessary to maintain normal serum concentrations of progesterone and luteal weights in sheep (Haworth et al., 1998). An additional pituitary hormone may also be necessary for normal luteal function during the mid-luteal phase, as hypophysectomy decreased concentrations of progesterone in sera and luteal weights more severely than specific removal of LH with an antiserum (Haworth et al., 1998). Both specific
removal of LH and hypophysectomy decreased luteal weight and luteal concentrations of mRNAs encoding StAR, P450scc and 3β-HSD (Haworth et al., 1998). Thus, removal of the pituitary decreases both the amount of luteal tissue and the capability of that tissue to synthesize progesterone. However, since hypophysectomy during the mid-luteal phase only reduced concentrations of progesterone in sera 4 days later by approximately 60% (Haworth et al., 1998), it seems likely that the corpus luteum of the ewe is somewhat independent of pituitary support at this time.

**Cellular regulation of enhanced synthesis of progesterone**

In small luteal cells, LH-induced increases of progesterone synthesis were associated with a slight increase in release of cholesterol from cholesterol esters but not with increased uptake of cholesterol or activity of P450scc or 3β-HSD (Wiltbank et al., 1993). However, the modest increase in cholesterol esterase activity was not sufficient to account for the marked increase in progesterone secretion in these cells. Therefore, it was postulated that LH was increasing transport of cholesterol through the cell or across the mitochondrial membranes. Since STAR facilitates the transport of cholesterol to P450scc (Stocco and Clark, 1996), regulation of this molecule appears to be crucial in control of secretion of progesterone. Chronic removal of LH decreases concentrations of mRNA encoding StAR (Juengel et al., 1995a); however, whether LH acutely increases luteal concentrations of StAR mRNA is not clear. Intra-ovarian infusion of LH did not increase steady-state concentrations of mRNA encoding STAR 4, 12 or 24 h later (Juengel et al., 1997a); thus, if LH acutely increases luteal concentrations of STAR mRNA it must do so in a very rapid and transient manner. However, a direct effect of activation of PKA on transcription of the StAR gene has been demonstrated as the human (Sugawara et al., 1997), mouse (Caron et al., 1997) and sheep (J. L. Juengel, C. M. Clay and G. D. Niswender; unpublished observations) STAR promoters respond directly to PKA activation with modest increases in activity. In addition, the ability of Star to stimulate steroidogenesis was increased by PKA-dependent phosphorylation (Arakane et al., 1997). Thus, the acute luteotrophic effects of LH are probably due to increased transcription, translation or phosphorylation of StAR.

Other hormones such as PGE2 (Fitz et al., 1984; Alila et al., 1988b), PGI2 (Fitz et al., 1984; Alila et al., 1988b), GH (Liebermann and Schams, 1994) and IGF-I (McArdle and Holtorf, 1989) have also been shown to increase secretion of progesterone from luteal cells; however, the mechanisms by which these hormones stimulate progesterone synthesis are not known. Prostaglandin E2 and I have been reported to increase cAMP (Marsh, 1975; Bennegard et al., 1990) and thus, in small luteal cells, would be expected to increase progesterone secretion in a manner similar to LH. However, both of these hormones also increase progesterone synthesis in large luteal cells (Fitz et al., 1984; Alila et al., 1988b), which do not respond to increased cAMP with an increase in progesterone secretion (Fitz et al., 1984; Hoyer et al., 1984). Therefore, binding of these hormones to receptors in large luteal cells must regulate progesterone secretion in another manner. A better understanding of how both basal and luteotropin stimulated progesterone synthesis is controlled in small, and particularly, large luteal cells is crucial if these processes are to be manipulated effectively.

**Cellular regulation of reduced secretion of progesterone**

Progesterone secretion is maintained for several days, and then rapidly declines if pregnancy does not occur. This rapid decline in the concentration of progesterone in sera is followed by a slower decrease in luteal weight. Prostaglandin F2α induces both the rapid decrease in progesterone secretion and loss of luteal tissue (Niswender and Nett, 1994). Binding of PGE2 to its receptor causes activation of PKC and an influx of calcium (Willbank et al., 1991). Pharmacological activation of PKC in vivo decreases secretion of progesterone without causing luteal cell death (McGuire et al., 1994). Similarly, a low dose of PGE2α, which transiently decreased secretion of progesterone and induced oligonucleosome formation, does not cause luteolysis (J. L. Juengel, J. D. Haworth, M. K. Rollyson, P. J. Silvia, E. W. McIntush, H. R. Sawyer and G. D. Niswender, unpublished observations). Thus, destruction of the corpus luteum is not required for reduced progesterone secretion from this tissue.
In fact, it seems clear that the anti-steroidogenic effects of PGF₂₅ are mediated via the PKC second messenger pathway, while the cytotoxic effects are mediated via calcium influx (Wiltbank et al., 1991).

Some mechanisms whereby activation of PKC by PGF₂₅ could decrease synthesis of progesterone at the cellular level include (1) influencing uptake or transport of cholesterol, (2) regulating conversion of cholesterol to pregnenolone or pregnenolone to progesterone, or (3) reducing the ability of the corpus luteum to respond to luteotrophic hormones by reducing receptors for LH, GH or both hormones, or interfering with the ability of the hormone receptor complex to activate their second messenger systems. The cellular mechanisms by which PGF₂₅ decreases synthesis of progesterone are rapidly being elucidated.

Treatment of ewes with PGF₂₅ decreased concentrations of mRNA encoding LDL-R; however, concentrations of HDL-BP mRNA actually increased during the first 12 h after treatment with PGF₂₅ (Fig. 3; Tandeski et al., 1996). Suppression of LDL-R mRNA to values similar to those seen after treatment with PGF₂₅ did not affect secretion of progesterone (T.R. Tandeski, J. L. Juengel, and G. D. Niswender, unpublished observations). Therefore, it seems unlikely that suppression of LDL-R mRNA is important for reduced secretion of progesterone during luteolysis. Furthermore, lipoprotein uptake was not limiting progesterone secretion in cultures of either ovine or bovine luteal cells after treatment with PGF₂₅ (Grusenmeyer and Pate 1992; Wiltbank et al., 1993). Finally, treatment of ovine luteal cells with PGF₂₅ did not affect release of cholesterol from cholesterol esters (Wiltbank et al., 1993). Thus, PGF₂₅ does not appear to reduce synthesis of progesterone by decreasing the availability of cholesterol for steroidogenesis.

In cultures of ovine and bovine luteal cells, PGF₂₅ appeared to decrease transport of cholesterol through the cytoplasm, or from the outer to inner mitochondrial membrane, or both (Grusenmeyer and Pate 1992; Wiltbank et al., 1993). Transport of cholesterol through the cell to the mitochondria is facilitated by the cytoskeleton (Jefcoate et al., 1992) and disruption of the cytoskeleton will decrease secretion of progesterone from luteal cells (Niswender and Nett, 1994). Treatment of ewes with PGF₂₅ rapidly disrupts the microtubular network of ovine corpora lutea (Murdoch, 1996). This disruption occurred before concentrations of progesterone in sera or luteal tissues decreased (Murdoch, 1996). In addition, in rat corpora lutea, administration of PGF₂₅ rapidly decreases concentrations of sterol carrier protein-2 (SCP-2), which facilitates transport of cholesterol through the cytoplasm (McLean et al., 1995). Whether PGF₂₅ has a similar effect on SCP-2 in luteal tissue of domestic ruminants is not known. Thus, one mechanism that PGF₂₅ may use to reduce progesterone synthesis is disruption of cholesterol transport to the mitochondria.

Prostaglandin F₂₅ also appears to disrupt transport of cholesterol across the mitochondrial membranes, potentially through regulation of StAR. Concentrations of StAR mRNA or protein in ovine and bovine corpora lutea were reduced within 12 h of treatment with PGF₂₅ (Fig. 3; Juengel et al., 1995a; Pescador et al., 1996). In addition, since the activity of StAR is modified by phosphorylation (Arakane et al., 1997), and StAR contains several potential PKC phosphorylation sites (Hartung et al., 1995; J. L. Juengel and G. D. Niswender, unpublished observations), its activity may be modified by PGF₂₅ to reduce progesterone synthesis more rapidly. Of particular interest are three potential PKC phosphorylation sites in the mitochondrial targeting sequence, the modification of which could be proposed to prevent targeting of StAR to the mitochondria. However, removal of the mitochondrial targeting sequence did not appear to affect the ability of StAR to stimulate steroidogenesis (Arakane et al., 1996). It should be pointed out that marked over-expression of the mutated form of StAR without its mitochondrial targeting sequence may have saturated the normal cholesterol transport mechanisms. Thus, the potential for modifications of the mitochondrial targeting sequence of StAR to interfere with cholesterol transport is unclear. The PBR system has been shown to be essential for transport of cholesterol to P450scc in other steroidogenic cells (Papadopoulus et al., 1997b); however, little is known about its potential regulation in luteal tissue of domestic ruminants. Thus, whether PGF₂₅-induced downregulation of progesterone synthesis is mediated partially through regulation of the PBR system remains to be determined. It is clear that one of the mechanisms by which PGF₂₅ decreases synthesis of progesterone in luteal cells is disruption of transport of cholesterol across the mitochondrial membranes.

For some time, conversion of cholesterol to pregnenolone was thought to be the rate-limiting
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Fig. 3. Pattern of expression of mRNA encoding high density lipoprotein-binding protein (HDL-BP), low density lipoprotein-receptor (LDL-R), luteinizing hormone-receptor (LH-R) and prostaglandin F$_2$-$
\alpha$-receptor (PGF$_{2\alpha}$-R) 4, 12 and 24 h after injection of PGF$_{2\alpha}$ are shown in the left panel. Values are expressed as a percentage of the control value. Pattern of expression of mRNA encoding steroidal acute regulatory protein (StAR), cytochrome P450 side-chain cleavage enzyme (P450scc) and 3β-hydroxysteroid dehydrogenase/Δ5,Δ4 isomerase (3β-HSD) as well as 3β-HSD enzyme activity (EA) after PGF$_{2\alpha}$ administration are given in the right panel. Within a variable, values that differ (P < 0.05) from controls (time of injection) are indicated with an asterisk. Data obtained from Guy et al., 1995; Juengel et al., 1995a, 1996, 1998; and Tandeski et al., 1996.

step in steroid synthesis. However, the activity, protein concentration and concentrations of mRNA encoding P450scc are not acutely reduced following treatment with PGF$_{2\alpha}$ (Fig. 3; Grusenmeyer and Pate, 1992; Wiltbank et al., 1993; McGuire et al., 1994; Tian et al., 1994; Rodgers et al., 1995). Thus, reduced progesterone synthesis is not due to a decreased capacity to convert cholesterol to pregnenolone. Induction of luteolysis with PGF$_{2\alpha}$ rapidly decreased mRNA encoding 3β-HSD in both ewes and cows (McGuire et al., 1994; Tian et al., 1994). However, in the same tissue that had markedly less mRNA encoding 3β-HSD, amounts of 3β-HSD protein or enzyme activity were not reduced (Fig. 3; Rodgers et al., 1995; Juengel et al., 1998). Thus, PGF$_{2\alpha}$-induced downregulation of luteal progesterone secretion does not appear to be mediated by decreased ability to convert pregnenolone to progesterone.

Administration of PGF$_{2\alpha}$ did not decrease concentrations of mRNA encoding GH-R or IGF-I (Juengel et al., 1997b). In fact, IGF-I mRNA and protein increased during the later stages of luteolysis (Perks et al., 1995). Binding of IGF-I to luteal tissue has also been shown to increase during luteolysis; however, this appeared to be due to increased expression of IGF-I binding proteins and not to an increased number of IGF-I receptors (Perks et al., 1995). Since IGF-I binding proteins can decrease IGF-I availability, decreased free IGF-I may be important in luteolysis. Injection of PGF$_{2\alpha}$ decreased mRNA encoding LH-R (Fig. 3; Guy et al., 1995); however, numbers of LH receptors were not decreased until after concentrations of progesterone in sera had declined (Diekman et al., 1978b; Spicer et al., 1981). Thus, the decrease in progesterone secretion following PGF$_{2\alpha}$ is not likely due to an inability of the corpus luteum to respond to tropic hormonal stimuli; however, PGF$_{2\alpha}$ could affect the ability of the hormone-receptor complex to activate its second messenger. Indeed, PGF$_{2\alpha}$ decreases adenylate cyclase activity and increases phosphodiesterase, thus decreasing the amount of cAMP available to activate PKA (Agudo et al., 1984; Carverick et al., 1985) which would decrease secretion of progesterone particularly from small luteal cells.

One of the most interesting aspects of the changes that occur after PGF$_{2\alpha}$ treatment of ewes is the marked decline in concentrations of mRNA encoding 3β-HSD, StAR, LDL-R, LH-R and PGF$_{2\alpha}$-R
Fig. 4. Potential mechanisms by which LH and prostaglandin $F_2_\alpha$ (PGF$_{2\alpha}$) regulate steroidogenic acute regulatory protein- (StAR) facilitated transport of cholesterol to cytochrome P450 side-chain cleavage enzyme (P450scc). Binding of LH to its receptor activates protein kinase A (PKA), which increases mRNA encoding StAR through transcriptional activation of the StAR gene but not through stabilization of the StAR mRNA (Kiriakidou et al., 1996). Increases in mRNA encoding StAR, and potentially increased translational efficiency of the StAR mRNA, would increase the amount of StAR protein. In addition, PKA phosphorylates StAR thereby increasing its ability to transport cholesterol to cytochrome P450scc. The anti-steroidogenic effects of PGF$_{2\alpha}$ have been shown to be mediated through activation of protein kinase C (PKC). Prostaglandin $F_2_\alpha$ decreases steady-state concentrations of mRNA encoding StAR, potentially by decreasing transcription of the StAR gene, destabilizing the StAR mRNA, or by both mechanisms. Concentrations of StAR protein decrease and it is possible that activity of the StAR protein is reduced through post-translational modifications, such as phosphorylation, resulting in decreased ability to bind cholesterol or decreased StAR-facilitated transport of cholesterol to P450scc. In addition, activation of PKC decreases cAMP concentrations, thus potentially reducing the PKA-activated increases in StAR activity. LDL-R: low density lipoprotein receptor; HDL-BP: high density lipoprotein-binding protein; 3β-HSD: 3β-hydroxysteroid dehydrogenase/Δ5,Δ4 isomerase.

while concentrations of mRNA encoding HDL-BP and P450scc were unaffected (Fig. 3). This may indicate that a very specific mechanism is activated by the PKC second messenger pathway which results in enhanced degradation or inhibited synthesis of some mRNAs with no effect on others. This mechanism may be specific to luteal cells as activation of PKC in other steroidogenic cell types has very different effects on several of the mRNAs encoding steroidogenic proteins.

Conclusion

Development and maintenance of the steroidogenic machinery in luteal cells is dependent upon pituitary hormones, LH and GH. These hormones not only support increases in luteal weight but
also support normal chronic expression of STAR, P450scc and 3β-HSD mRNA, which encode three proteins essential for progesterone synthesis. Luteotrophic and luteolytic hormones also acutely regulate progesterone synthesis. The primary effect of LH appears to be regulation of pregnenolone production by increasing transport of cholesterol to cytochrome P450scc. One protein likely to facilitate this process is STAR. The precise mechanisms by which STAR facilitates transport of cholesterol to cytochrome P450scc are not known. However, phosphorylation of STAR by PKA is likely one mechanism by which LH acutely enhances the activity of Star and thus progesterone synthesis (Fig. 4). In addition, activation of PKA may also increase transcription of the STAR gene, or increase translation efficiency of STAR mRNA (or both), thereby increasing the amount of STAR protein available to facilitate transfer of cholesterol (Fig. 4). Prostaglandin F2α reduces transport of cholesterol across the mitochondrial membranes, potentially by decreasing amounts of STAR protein or its ability to transport cholesterol. Prostaglandin F2α may reduce STAR protein by decreasing transcription of the STAR gene, interfering with stability of the STAR transcript or reducing translation efficiency of the STAR mRNA (Fig. 4). Once STAR is inserted into the mitochondria, it is likely that it is no longer able to transport cholesterol. Therefore, only newly synthesized protein transports cholesterol giving STAR an effective half life of 3–5 min. Thus, one mechanism that both luteotropic and luteolytic hormones appear to use to regulate progesterone secretion from the corpus luteum acutely is regulation of STAR-facilitated transport of cholesterol. This may be accomplished through regulation of transcription of the STAR gene, stability of the STAR mRNA, synthesis of STAR protein, or post-translational modifications of STAR such as phosphorylation (Fig. 4).

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