Mechanisms Controlling the Function and Life Span of the Corpus Luteum

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Niswender, Gordon D., Jennifer L. Juengel, Patrick J. Silva, M. Keith Rollyson, and Eric W. McIntush. Mechanisms Controlling the Function and Life Span of the Corpus Luteum. Physiol. Rev. 80: 1–29, 2000.—The primary function of the corpus luteum is secretion of the hormone progesterone, which is required for maintenance of normal pregnancy in mammals. The corpus luteum develops from residual follicular granulosal and thecal cells after ovulation. Luteinizing hormone (LH) from the anterior pituitary is important for normal development and function of the corpus luteum in most mammals, although growth hormone, prolactin, and estradiol also play a role in several species. The mature corpus luteum is composed of at least two steroidogenic cell types based on morphological and biochemical criteria and on the follicular source of origin. Small luteal cells appear to be of thecal cell origin and respond to LH with increased secretion of progesterone. LH directly stimulates the secretion of progesterone from small luteal cells via activation of the protein kinase A second messenger pathway. Large luteal cells are of granulosal cell origin and contain receptors for PGF$_{2\alpha}$ and appear to mediate the luteolytic actions of this hormone. If pregnancy does not occur, the corpus luteum must regress to allow follicular growth and ovulation and the reproductive cycle begins again. Luteal regression is initiated by PGF$_{2\alpha}$ of uterine origin in most subprimate species. The role played by PGF$_{2\alpha}$ in primates remains controversial. In primates, if PGF$_{2\alpha}$ plays a role in luteolysis,
it appears to be of ovarian origin. The antisteroidogenic effects of PGF\textsubscript{2\alpha} appear to be mediated by the protein kinase C second messenger pathway, whereas loss of luteal cells appears to follow an influx of calcium, activation of endonucleases, and an apoptotic form of cell death. If the female becomes pregnant, continued secretion of progesterone from the corpus luteum is required to provide an appropriate uterine environment for maintenance of pregnancy. The mechanisms whereby the pregnant uterus signals the corpus luteum that a conceptus is present varies from secretion of a choric gonadotropin (primates and equids), to secretion of an antiluteolytic factor (domestic ruminants), and to a neuroendocrine reflex arc that modifies the secretory patterns of hormones from the anterior pituitary (most rodents).

I. INTRODUCTION

A. Historical Perspective

Corpora (bodies) lutea (yellow) were named by Marcello Malpighi (1628–1694) and first accurately described by Regnier de Graaf (1641–1673) who noted that after coitus “globular bodies” appeared on the ovary of rabbits and remained there until after parturition. De Graaf also observed that the number of corpora lutea was related to the number of offspring that animals “bring forth” (as cited by Short, Ref. 332). That the corpus luteum might produce substances that regulate pregnancy was suggested by Prenant (293) after examining the histology of corpora lutea and concluding that “there can be no doubt . . . it (the corpus luteum) acts as a gland, and as a gland of internal secretion . . .” Subsequently, Prenant’s conclusion was confirmed when it was shown that removing the ovaries or corpora lutea from pregnant rabbits resulted in abortion or resorption of the embryos (112, 113, 208). After these experiments, Magnus (208) suggested treating pregnant, ovariectomized rabbits with luteal extracts to study the biologically active factor produced by corpora lutea. Thereafter, the luteal factor was crystallized and characterized virtually simultaneously by four independent groups (7, 49, 142, 339). This factor, which proved to be a steroid hormone, was named progesterone.

B. General Actions of Progesterone

Although the focus of this review is regulation of the synthesis and secretion of progesterone by the corpus luteum, a brief introduction regarding the biological actions of this important reproductive hormone is merited. The principal targets of progesterone are the reproductive tract and the hypothalamo-pituitary axis. In general, the actions of progesterone on the reproductive tract are to prepare it for initiation and maintenance of pregnancy. Progesterone appears to exert most of its effects by directly regulating transcription of genes through specific nuclear receptors that act as ligand-inducible transcription factors (reviewed in Moutsatsou and Sekeris, Ref. 239). Upon ligand binding, these receptors modulate expression of genes by binding specific progesterone-responsive elements on the DNA. Previous exposure to estrogens, which induces the production of receptors for progesterone (167, 182, 192), is required for progesterone to act on the reproductive tract. In contrast, progesterone downregulates receptors for estradiol (46, 94, 168, 383) and thereby blocks many of the actions of estrogens that generally act as mitogenic factors. An example of the antiestrogenic effects of progesterone is in the oviduct where progesterone blocks estradiol-inducible secretory proteins (375) and induces deciliation and cessation of secretory activity of the oviductal epithelium (45, 46, 322).

In the uterus, progesterone acts on the endometrium as a differentiation factor (70). During the follicular phase, estrogens induce proliferation of cells of the endometrium, and elevated concentrations of progesterone during the luteal phase of the reproductive cycle inhibit mitosis in the endometrium (269). Progesterone also induces stromal differentiation, stimulates glandular secretions in association with the accumulation of basal vacuoles in the glandular epithelium (216), and changes the pattern of proteins secreted by endometrial cells (216, 355). These uterine proteins provide an environment that supports early embryonic development.

In the uterus, progesterone induces quiescence of the myometrium. This effect appears to be manifested in an increased resting potential and prevention of electrical coupling between myometrial cells (273). In addition, progesterone decreases uptake of extracellular calcium that is required for contraction of myometrial cells (26) by downregulating expression of genes that encode subunits of voltage-dependent calcium channels (362). Progesterone also prevents uterine contractions by blocking the ability of estradiol to induce \(\alpha\)-adrenergic receptors, activation of which causes contractions (37).

Finally, the length of reproductive cycles is also governed, in part, by progesterone. Circulating concentrations of progesterone are low during the follicular phase. During this time, rising concentrations of estradiol act on the hypothalamus and pituitary to stimulate low-amplitude, high-frequency pulses of luteinizing hormone (LH), which result in elevated circulating concentrations of LH that drive follicular development to the point of ovulation (reviewed in Lucy et al., Ref. 205). After ovulation, as the corpus luteum develops, high circulating concentrations of progesterone restrict secretion of LH to low-frequency,
high-amplitude pulses that result in reduced mean concentrations of LH. This effect of progesterone is the result of actions on both the hypothalamus and pituitary. Progesterone blocks surges of gonadotropin-releasing hormone (GnRH) from the hypothalamus (17, 183). In the pituitary, progesterone reduces the number of receptors for GnRH (198) by downregulating mRNA encoding the receptor for GnRH (27). In addition, progesterone decreases the amount of LH released in response to GnRH (170), in part as a result of the reduced number of receptors for GnRH in the pituitary. High levels of progesterone also result in decreased expression of the genes encoding the β-subunits of both LH (42) and follicle-stimulating hormone (FSH) (42, 87) and the common α-subunit of the gonadotropins (18, 42, 87). These effects of progesterone on secretion of gonadotropins appear to be dependent on the total endocrine environment because, in some instances, progesterone can facilitate surges of gonadotropins induced by estradiol (17, 27, 40, 41, 193).

II. DEVELOPMENT OF THE CORPUS LUTEUM

This review of the mechanisms that control luteal function focuses on domestic ruminants, and data from the ewe are provided for continuity in essentially all areas of discussion. Data from additional species are included where appropriate. A second area of focus is the control of the secretion of progesterone at the molecular level, again with particular emphasis on the ewe.

The preovulatory surge of gonadotropins induces ovulation and differentiation of residual follicular cells that form the corpus luteum and begin to produce progesterone at high rates. Before this, estradiol is the primary steroid secreted by the ovary. Granulosal and thecal cells of the follicle coordinately produce estrogens (reviewed in Refs. 24, 111). Thecal cells express the enzymes necessary to convert cholesterol to androgens but lack the enzymes necessary to convert androgens to estradiol (reviewed in Bao and Garverick, Ref. 24). Conversely, granulosal cells produce progesterone but are unable to convert pregnenolone or progesterone to androgens. However, granulosal cells can convert androgens to estradiol. Thus thecal cell-produced androgens are aromatized to estradiol by granulosal cells. Estradiol is important as a mitogen and stimulates the division of granulosal cells (306). The preovulatory LH surge results in luteinization of granulosal and thecal cells and alters the steroidogenic pathway so that progesterone is the primary steroid hormone produced by each of these cell types after luteinization. However, the corpus luteum of several species, including humans, pigs, and rats, retains the ability to produce some estradiol (reviewed in Refs. 305, 393, 394). Synthesis of progesterone is the least complex steroidogenic pathway in the ovary (Fig. 1). Differentiation into cells capable of producing progesterone at high rates is accomplished by increased expression of enzymes necessary for conversion of cholesterol to progesterone, i.e., cholesterol side-chain cleavage cytochrome P-450 complex (P-450_{scc}), and 3β-hydroxysteroid dehydrogenase/Δ5,Δ4 isomerase (3β-HSD), and decreased expression of the enzymes that convert progesterone to estrogens, i.e., 17α-hydroxylase cytochrome P-450 and aromatase cytochrome P-450 (reviewed in Bao and Garverick, Ref. 24).

Theca- and granulosa-derived luteal cells give rise to two distinct types of luteal cells that differ morphologically and physiologically. In most nonprimate mammals, the cells derived predominantly from granulosal cells have been designated as large luteal cells (LLC), and those from thecal cells have been designated small luteal cells (SLC). The primate analogs of LLC and SLC are referred to as granulosa-lutein and theca-lutein cells, respectively (254). Differences in the morphological and biochemical characteristics of these cell types, including differences among species, are detailed in section IV.
addition to steroidogenic cells, the corpus luteum contains endothelial cells, fibroblasts, pericytes, and cells originating from the bloodstream (59, 60).

The degree of migration and intermixing of follicular-derived cells during formation of the corpus luteum differs among species. In primates, migration and intermixing are less extensive than in other species. Granulosa-lutein cells remain separated from theca-lutein cells, and some of the follicular basement membrane appears to remain and form a barrier between granulosa-lutein and theca-lutein cells (132). In contrast, follicular tissue is extensively reorganized during migration of thecal cells, fibroblasts, and endothelial cells during development of the corpus luteum of most nonprimate mammals (254). In these species, the cells of the corpus luteum are intermixed to the extent that LLC, SLC, fibroblast, and endothelial cells are in close proximity to one another (Fig. 2; Refs. 84, 99).

A profound aspect of early luteal development is the rate of tissue growth and cellular proliferation during this time. In sheep, ovulatory follicular tissue that weighs ~40 mg develops into a corpus luteum that weighs 600–700 mg in just a few days (99, 169). This growth is the result of an approximately twofold increase in the size of LLC, whose numbers remain constant (~15 × 10^6/corpus luteum in sheep), and an increase in the number of SLC, fibroblasts, and endothelial cells (99). From days 4 to 16 of the ovine estrous cycle, the number of fibroblasts approximately doubles (from 21 to 50 × 10^6), whereas the number of SLC increases 5-fold (from 10 to 50 × 10^6) and the number of endothelial cells increases ~6.5-fold (from 18 to 120 × 10^6; Ref. 99). Proliferation of cells in the developing corpus luteum results in a mitotic rate that is equal to that of rapidly growing tumors (169). The factors...
regulating proliferation of SLC and fibroblasts are not well characterized but may involve fibroblast growth factors (300), growth hormone (GH; Ref. 179), and LH (129). Vascular endothelial growth factor (VEGF), a mitogen specific for endothelial cells (102), is probably a primary regulator of proliferation of luteal endothelial cells early in the cycle (29). Luteinizing hormone or human chorionic gonadotropin (hCG) induces expression of VEGF by preovulatory follicles and isolated granulosal cells (116, 188), and immunoneutralization of VEGF abolishes mitogenic activity of endothelial cells in developing corpora lutea (89).

Proliferation of endothelial cells is requisite for the neovascularization during luteal development that results in the corpus luteum’s extensive capillary network (300, 304). Capillary lumina account for 22% of the total volume of the corpus luteum (84), which is consistent with a rate of blood flow (6–10 ml·g⁻¹·min⁻¹) to the corpus luteum that exceeds that of other tissues. In addition, most membranes of luteal cells are either directly adjacent to capillaries (50%) or adjacent to the interstitial space (37%) in close proximity to capillaries (84). Such juxtapositioning of luteal cells to capillaries provides for the high metabolic demands of corpora lutea, which consume two to six times more oxygen per unit weight than does the liver, kidney, or heart (357).

### III. LUTEAL STEROIDOGENIC PATHWAY

#### A. Steroidogenic Substrates

The substrate for steroidogenesis is cholesterol (Fig. 1). Under normal conditions, the majority of cholesterol is synthesized in the liver (194) and transported to steroidogenic tissues such as the adrenal cortex, follicle, corpus luteum, and testis in the form of lipoproteins. Low-density lipoprotein (LDL) and high-density lipoprotein (HDL) are the most common sources of cholesterol for the production of steroid hormones by the corpus luteum (165, 261, 277). There appears to be some species differences in their preference for LDL or HDL, but either source can be utilized by luteal cells of most species. Interestingly, in sheep, maximal stimulation of progesterone secretion by lipoprotein correlates well with normal levels of HDL or LDL in serum (387). In addition, under conditions of lipid deprivation (reduced lipoprotein synthesis or in most in vitro conditions), luteal cells are capable of synthesizing cholesterol from acetate (67, 68, 181). However, under normal conditions, the vast majority of cholesterol used for steroidogenesis is obtained from the bloodstream in the form of LDL or HDL.

The uptake of LDL by luteal cells occurs by receptor-mediated endocytosis (47). This pathway is quite efficient, since each LDL molecule contains ~2,500 cholesterol molecules. Once internalized, the endosomes combine with lysosomes where the LDL dissociates from the receptor and is broken down making free cholesterol available to the cell. The LDL receptor is recycled or degraded (reviewed in Grummer and Carroll, Ref. 130). Uptake of extracellular HDL occurs after binding to a plasma membrane-bound HDL binding protein, and cholesterol is transported into the cell by an undefined mechanism that does not appear to be receptor-mediated endocytosis (reviewed in Lestavel and Fruchart, Ref. 201).

Once free cholesterol is present in the cytosol of the cell, it can be used for steroidogenesis or formation of cell membranes, or it can be esterified with fatty acids to form cholesterol esters by cholesterol ester synthetase and stored (reviewed in Johnson et al., Ref. 172). The cholesterol esters often form lipid droplets that have long been used as a morphological characteristic of steroidogenic cell types. Cholesterol esterase hydrolyzes the stored cholesterol esters and provides free cholesterol for use by the cell. This is one of the first steps in steroidogenesis that is acutely controlled by second messenger pathways. Cholesterol esterase is activated when phosphorylated by protein kinase A (PKA) (51, 286, 367).

#### B. Transport of Cholesterol

Synthesis of all steroids is dependent on transport of cholesterol to the mitochondria and then from the outer to the inner mitochondrial membrane where the cholesterol side-chain cleavage enzyme complex cleaves the side chain from cholesterol to form pregnenolone (354). The first step in this process, transport to the outer mitochondrial membrane, appears to require an intact cytoskeleton, since inhibitors of both microtubule and microfilament (69) function prevent mitochondrial accumulation of cholesterol. The phosphorylation status of cytoskeletal proteins likely influences the rate of steroid transport. Sterol binding proteins also appear to play a role in the transport of cholesterol to the mitochondria (166; reviewed in Scallen et al., Ref. 323). Stimulation of steroidogenesis by tropic hormones enhances transportation of cholesterol to mitochondria, which appears to be a complicated process that is not explained by simple diffusion of cholesterol through the cytoplasm.

The rate-limiting step in the steroidogenic pathway appears to be transport of cholesterol from the outer to the inner mitochondrial membrane (349). This step also appears to be the primary site of acute positive and negative regulation of steroidogenesis by second messenger systems (337). It has been known for at least three decades that stimulation of the steroidogenic pathway by tropic hormones requires the synthesis of a short-lived protein. A breakthrough recently occurred when two proteins of similar size (30,000 M₀) were identified whose
synthesis was associated with increased steroid production in several tissues after hormonal stimulation (195, 289, 290, 350, 351). Phosphorylation of these proteins was associated with further increases in steroid secretion (92, 289). The proteins localized to mitochondria and were generated by posttranslational modifications of a protein of 37,000 M_r that contained a mitochondrial targeting sequence (64, 353). This protein, named steroidogenic acute regulatory protein (StAR), was cleaved to acidic and basic 30,000 M_r forms during insertion into the mitochondrial membrane. It was hypothesized that it was during insertion of these proteins into the mitochondrial membrane that cholesterol was transported to the enzyme complex that cleaves the side chain from cholesterol to form pregnenolone (381). However, more recent evidence obtained by truncation of the STAR gene to delete the mitochondrial targeting sequence suggests that cholesterol transport also occurred with the mutated protein (12, 378). Therefore, the role of the mitochondrial targeting sequence in cholesterol transport is unclear. The critical role played by StAR in steroidogenesis was clearly documented by Lin et al. (203), who demonstrated that the severely reduced adrenal and gonadal steroid synthesis seen in patients with congenital lipoid adrenal hyperplasia was due to naturally occurring mutations in the StAR gene.

Recently, the peripheral-type benzodiazepine receptor, which is present in the membranes of mitochondria in steroid-producing cells, has also been shown to play a role in transport of cholesterol (271). Targeted deletion of the gene for this receptor from cells that constitutively produce steroids results in a dramatic reduction in steroid secretion, which is reversed if the receptors are reintroduced into the cells (270). It has also been shown that normal levels of the endogenous ligand for this receptor are required for a normal steroidogenic response to hCG (38). Thus StAR, mitochondrial benzodiazepine receptors, and the endogenous ligand for this receptor all appear to be required for normal transport of cholesterol from the outer to the inner mitochondrial membrane, the site of cholesterol side-chain cleavage.

C. Conversion of Cholesterol to Progesterone

Once transported to the mitochondrial matrix, the actions of 3b-HSD convert pregnenolone to progesterone (354). Pregnenolone is then transported to the smooth endoplasmic reticulum, which is usually closely associated with mitochondria, where 3b-HSD converts pregnenolone to progesterone (reviewed in Hanukoglu, Ref. 140). Progesterone is then thought to diffuse from the cell. There is no evidence that progesterone can be stored in high quantities in luteal tissue.

Although progesterone is the primary steroid hormone secreted by the corpus luteum, depending on species and reproductive state, additional steroids may be secreted in significant quantities. For example, corpora lutea of cattle secrete 20b-hydroxy, preg 4-ene, 3-one, and 20a-hydroxy, preg 4-ene, 3-one is secreted by corpora lutea of many species late in the luteal phase of the estrous cycle. In addition, estrogens are produced and secreted by primate corpora lutea during the luteal phase of the menstrual cycle (50).

IV. TROPIC REGULATION OF LUTEAL FUNCTION

Luteotropic hormones are those that support the growth and/or function of the corpus luteum. During a normal luteal phase, the corpus luteum increases in size and ability to secrete progesterone (Fig. 3). Once the corpus luteum has obtained it mature size and reached its maximal potential for secretion of progesterone, luteal function is maintained for a few to several days depending on the species, and then if the animal does not become pregnant, luteal regression must occur to allow resumption and another chance for pregnancy to occur.

Concentrations of progesterone in serum are dependent on the amount of steroidogenic tissue, blood flow, and capacity of the steroidogenic tissue to synthesize progesterone. The amount of steroidogenic tissue is dependent on number, as well as size, of steroidogenic luteal cells, both of which increase during luteal development (Fig. 3). Blood flow to the corpus luteum also increases as concentrations of progesterone in serum increase. Increases in concentrations of progesterone in serum are also associated with changes in concentrations of mRNA encoding components of the luteal regulatory and/or steroidogenic pathway. Figure 3 depicts steady-state concentrations of mRNA encoding receptors for HDL, LDL, LH, PGF_2, and GH as well as insulin-like growth factor (IGF)-I during development (days 3 and 6), maintenance (days 9 and 12), and early regression (day 15) for the ovine corpus luteum. These are thought to be the key receptors that regulate cholesterol uptake or mediate the positive and negative effects of hormones on luteal secretion of progesterone. Figure 3 also contains data regarding mRNA concentrations encoding the major steroidogenic proteins, StAR, P-450 MCC, and 3b-HSD, during the same stages of luteal function. Although serum concentrations of progesterone increase as much as 20-fold and luteal weights increase 4-fold, there are no dramatic changes (more than 2-fold) in steady-state concentrations of any of the mRNA species except that encoding the receptor for LH, which increases 5-fold. However, if total mRNA per corpus luteum were calculated for any of the components of the steroidogenic pathway, there would be dramatic
increases for all mRNA species. It is important to point out that it is the concentration of each species of mRNA within an individual cell that is the key to controlling concentrations of important regulatory proteins such as receptors and enzymes and therefore is the key to regulation of the steroidogenic activities of that cell. Thus steroidogenic capacity of individual luteal cells, along with their ability to respond to the luteotropic hormone LH, increases during luteal development.

Many experimental approaches have been used to delineate the hormones necessary for normal luteal function. These have included classical ablation/replacement experiments, specific neutralization of a particular hormone with antibodies, suppression of secretion of a hormone with pharmacological agents, and the use of hormone-specific antagonists. In addition, examination of the effects of specific hormones on progesterone production from isolated luteal cells has allowed identification of potential luteotropic substances and given us further insight as to how luteotropic hormones may regulate progesterone secretion. Hormones that support the growth and/or function of the corpus luteum are termed luteotropic hormones and include LH, GH, prolactin, IGF-I, oxytocin, PGE$_2$, and PGI$_2$.

A. Luteal Development

The importance of hormones secreted by the pituitary gland for normal luteal development and function has been demonstrated in many species (15, 81, 100, 164, 343, 348, 374). For continuity, much of this discussion focuses on the role of the pituitary in regulating luteal function in ewes. If the pituitary is removed on day 5 of the estrous cycle, the corpus luteum does not continue to increase in weight, and concentrations of progesterone in serum remain at or below levels observed on day 5 (100, 179). This loss of luteal weight, when compared with that in intact control ewes, is associated with a decrease in the number of SLC and fibroblasts and a decrease in the size of both small and large steroidogenic luteal cells (100). The decrease in amount of progesterone in serum does not appear to be associated with decreased uptake of lipoproteins as concentrations of mRNA encoding LDL-receptor (R) or the HDL-binding protein (BP), and presumably their proteins, are not decreased (360). However, removal of the pituitary is followed by reduced levels of mRNA encoding the steroidogenic proteins StAR, P$_4$-450scc, and 3$eta$-HSD (177, 179). The decrease in the number and size of steroidogenic cells and the decreased capacity for steroidogenic cells to synthesize progesterone result in reduced ability of the corpus luteum to secrete progesterone.

Hypophysectomy removes all pituitary hormones from the circulation; however, in the ewe, physiological replacement of LH and GH is adequate to support normal luteal development. Physiological (pulsatile) replacement of LH in the ewe hypophysectomized during luteal development supports normal progesterone secretion and nor-
mRNA encoding StAR, P-450\(_{scC}\), and 3\(\beta\)-HSD; however, luteal weights remain lower than in control animals (100, 177, 179). The failure to attain normal luteal weights is likely due to fewer nonsteroidogenic cells because the number and size of steroidogenic cells is different from those observed in control ewes. Treatment of hypophysectomized ewes with GH alone also allows circulating concentrations of progesterone and expression of mRNA encoding StAR and P-450\(_{scC}\) to reach normal levels; however, GH does not appear to support luteal expression of mRNA encoding 3\(\beta\)-HSD (177, 179). Replacement of GH in hypophysectomized ewes increases luteal weights over those in untreated, hypophysectomized ewes, but not to the size observed in pituitary-intact control ewes (179). Only when both LH and GH were replaced in hypophysectomized ewes did all parameters of luteal function measured increase to levels that were indistinguishable from pituitary-intact control ewes. Thus both LH and GH are necessary for normal luteal development and function in ewes.

**B. Chronic Regulation of Luteal Function by Pituitary Hormones: Luteal Maintenance**

After the corpus luteum is fully formed in the ewe, removal of the pituitary results in regression of the corpus luteum (79, 81, 144). Although results from early studies suggest a role for prolactin in maintaining corpora lutea in hypophysectomized/hysterectomized ewes (81), suppression of prolactin secretion by ergocryptin does not affect luteal function in normally cycling ewes or cows (154, 250) or in pituitary-stalk disconnected (253) or hypophysectomized ewes that had been treated with LH (100). Thus prolactin does not appear to be essential for normal luteal function during the estrous cycle in cows and ewes. In contrast, in ewes, cattle, and pregnant but not cycling swine, treatment with antisera against LH caused a decline in luteal weight and/or luteal content of progesterone (115, 302, 343, 348). However, in ewes, the effects of removal of all pituitary hormones by hypophysectomy had a more severe effect on luteal function than did specific removal of LH with antiserum (144). Both treatments resulted in a decrease in concentrations of mRNA encoding StAR, P-450\(_{scC}\), and 3\(\beta\)-HSD (144). Whether this occurred before the decrease in concentrations of progesterone in sera was not determined. However, it seems clear that LH is required to maintain normal expression of mRNA, and presumably proteins, encoding StAR, P-450\(_{scC}\), and 3\(\beta\)-HSD. Thus both LH and GH appear to be necessary for normal development of the corpus luteum and for maintenance of the function of the mature corpus luteum in sheep.

A critical role for LH in supporting progesterone secretion in primates has also been demonstrated (135, 164, 374). Treatment of monkeys with a potent GnRH antagonist causes a dramatic decrease in concentrations of progesterone in serum, which was associated with decreases in mRNA encoding the steroidogenic enzymes P-450\(_{scC}\) and 3\(\beta\)-HSD (297). Therefore, expression of mRNA encoding proteins important for steroidogenesis, and presumably the proteins themselves, is dependent on LH.

**C. Requirements for Pulsatile Release of LH for Normal Luteal Function**

Luteinizing hormone is released from the pituitary gland in a pulsatile manner; however, whether the pulsatile profile of LH was essential for normal luteal development and function was unclear. The use of a GnRH antagonist, which obliterates pulsatile, but not basal, release of LH, has allowed researchers to address this question. In primates, administration of a GnRH antagonist prevents normal luteal development (114) and causes a rapid decline in secretion of progesterone from mature corpora lutea (136, 297, 382). However, in early studies in women who had undergone hypophysectomy, progesterone secretion could be maintained with a single, large dose of hCG (374). However, this result is difficult to interpret due to the prolonged circulating half-life of hCG. In cattle, treatment with GnRH antagonist during luteal development impairs normal function of the corpus luteum, indicating that pulsatile release of LH is necessary for this process (283). However, the effect on secretion of progesterone is not as dramatic as that observed in primates, and treatment of cows with a GnRH antagonist after the corpus luteum is fully developed had no effect on secretion of progesterone (283). Treatment of sheep during development or maintenance of the corpus luteum with GnRH antagonist had very little effect on the secretion of progesterone (225). Thus pulsatile release of LH appears necessary for normal luteal function in primates and for luteal development in cows. However, pulses of LH did not appear necessary for luteal development in ewes or maintenance of progesterone secretion in cows or ewes. Thus, although LH is luteotropic in most mammals, the requirement for pulsatile secretion of LH to support secretion of progesterone varies greatly. It should be pointed out that treatments that reduce pulsatile secretion of LH also reduce the average concentrations of LH; thus these data need to be interpreted with care.

**D. Role of Estrogens in Luteal Function**

In some species, such as the rat, rabbit, and pig, estrogens are luteotropic. However, the dependence of the corpus luteum on estradiol for support varies in these species. In the rabbit, removal of estradiol causes cessa-
tion of progesterone secretion; thus estradiol is considered the primary luteotropic hormone. In this species, LH plays a secondary role, that of stimulating estradiol synthesis in follicles (reviewed in Holt, Ref. 155). Similarly, in the rat, estradiol appears to be directly involved in stimulation of progesterone secretion. Although both prolactin and LH are necessary for luteal function in the rat, their roles are secondary. Prolactin is essential to maintain expression of estradiol and LH receptors, and LH stimulates synthesis of estradiol from the corpus luteum (reviewed in Gibori et al., Ref. 119). In both rats and rabbits, estrogens also stimulate, directly or indirectly, hypertrophy of luteal cells seen during pregnancy (reviewed in Refs. 119, 155). In the pig, the luteotropic role of estrogen is less defined. Implantation of estradiol capsules into the corpus luteum results in growth of the corpus luteum and increased progesterone secretion (65). Estradiol also reduces secretion of PGF$_{2\alpha}$ from the uterus, thus preventing luteolysis which may account for some of the effects of estradiol in the pig (110).

Another potential mechanism whereby luteotropic hormones could support luteal function is decreased expression of receptors for the luteolytic hormone PGF$_{2\alpha}$. However, LH appears to upregulate expression of mRNA encoding the PGF$_{2\alpha}$-R (180, 371), and hypophysectomy during the midluteal phase does not affect luteal concentrations of this mRNA (144). Thus luteotropic hormones do not appear to support luteal function by suppressing receptors for PGF$_{2\alpha}$.

E. Acute Control of Progesterone Secretion

The ability of hormones to acutely (within minutes to hours) increase secretion of progesterone in luteal tissues has been examined in vitro as well as in vivo. In vitro studies have utilized cultures of minced luteal tissue, dissociated luteal cells, and/or isolated cell types. The most critical information has been obtained from studies that evaluated steroidogenesis in the two separate types of steroidogenic cells present in corpora lutea from all species examined (reviewed in Niswender and Nett, Ref. 254). Small and LLC differ in their basal rates of secretion of progesterone, with LLC producing 2- to 40-fold more progesterone than unstimulated SLC. The two luteal cell types also differ in their response to different hormonal and/or second messenger stimuli (reviewed in Ref. 254).

1. Acute effects of LH on progesterone secretion

Because of the primary role LH plays in luteal development and function, its role and mechanisms of action in
acute stimulation of progesterone secretion from luteal cells have been examined. In the ewe (161, 310), cow (6), human (260), and pig (361), physiological concentrations of LH increase secretion of progesterone from SLC, but not LLC (Fig. 4), although in the ewe and the cow it has been shown that both cell types contain receptors for LH (62, 141). In ovine and bovine luteal tissues or cells, binding of LH to its receptor activates adenylate cyclase, leading to increased concentrations of cAMP and ultimately activation of PKA (74, 161, 213). Activation of PKA in SLC slightly increases release of cholesterol from cholesterol esters (386) but does not influence concentrations of mRNA or the activity of P-450scc or 3β-HSD (30, 386). The modest increase in cholesterol esterase activity is not sufficient to cause the 5- to 20-fold increase in progesterone secretion observed after treatment of SLC with LH (386). Thus the acute steroidogenic effects of LH do not appear to be modulated by acute changes in the three steroidogenic enzymes involved in biosynthesis of progesterone. Therefore, it was suggested that LH increases steroid production by facilitating transport of cholesterol through the cell and to P-450scc (Fig. 4; Ref. 386). An attractive candidate for LH regulation of progesterone synthesis is the peripheral benzodiazepine receptor (PBR)/endozepine system, which in other cell types is important for steroid synthesis (271). To date, there has been little research to examine the role of this system in luteal tissue. However, evidence is accumulating that StAR and the PBR/endozepine system likely interact in transporting cholesterol through the mitochondrial membranes. Other proteins, such as the voltage-dependent anion channel, may also be involved in this process (271).

Addition of LH to SLC has also been shown to activate phospholipase C (PLC) and presumably protein kinase C (PKC) (reviewed in Davis et al., Ref. 74). Whether or not LH causes activation of PLC appears to depend on methodological and not species differences because LH has been found to activate (74) or not activate (390) the PLC/PKC second messenger system in ovine SLC. One obvious difference between these studies is the use of suspended versus plated cells. Even within the same laboratory, activation of PLC/PKC activity by LH is found in suspended but not plated cells (74). Thus the process of plating SLC appears to cause a loss of PLC/PKC activation by LH. Whether this is due to a change in the cells upon plating or to loss of cells found in SLC preparations that do not plate is unknown. Ovine SLC preparations are ~50% pure (105) and contain endothelial cells, which do not plate under standard plating conditions. Luteinizing hormone binding sites have been found on bovine endothelial cells (62). Therefore, it is interesting to speculate
that LH may activate the PLC/PKC second messenger system preferentially in endothelial cells and the PKA second messenger system in small steroidogenic luteal cells. However, it is clear that pharmacological activation of PKA (forskolin) and PKC [phorbol 12-myristate 13-acetate (PMA)] in ovine small steroidogenic luteal cells has opposing actions on steroidogenesis (386). Thus having one hormone activate both second messenger systems in small steroidogenic luteal cells seems unlikely.

In nonhuman primates and pregnant or pseudopregnant rats, LLC in addition to SLC respond to LH with increases in progesterone secretion (43, 151, 245, 361). However, binding of LH to its receptor on LLC collected from ewes does not increase concentrations of intracellular cAMP, and addition of 8-bromo-cAMP or dibutyryl cAMP does not lead to increased secretion of progesterone (161). In addition, physiological concentrations of LH do not increase secretion of progesterone from human, bovine, or porcine LLC (6, 260, 361). However, these cells produce very high basal levels of progesterone (254), and it has been suggested that the PKA system may be constitutively activated in this cell type (161). Interestingly, it has been calculated that LLC produce >80% of total luteal progesterone secreted during the mid-luteal phase of the estrous cycle in the ewe (256). Thus understanding regulation of progesterone synthesis in this cell type is critical to allow manipulation of progesterone output from the corpus luteum.

2. Acute effects of GH and IGF-I on progesterone secretion

Both GH (202) and IGF-I (66, 82, 220, 274, 318) increased secretion of progesterone from luteal tissue. Receptors for GH or mRNA encoding GH receptor have been identified in ovine, bovine, and rat luteal tissue (53, 178, 204). Growth hormone could have a direct effect on luteal function through binding to its receptor and activation of the membrane-associated tyrosine kinase JAK2 (14). In support of this suggestion, perfusion of GH directly into the corpus luteum increased secretion of progesterone and oxytocin within 60 min (202). In addition, GH may influence luteal function indirectly by increasing expression of IGF-I, which is made by the corpus luteum of many species (mRNA and/or protein; Refs. 178, 259, 274, 281, 359). Indeed, in the ewe, GH supports IGF-I expression from luteal tissue (178). Receptors for IGF-I have been demonstrated in luteal tissues of several species including the cow, human, ewe, and rat (259, 274, 281, 318, 359). Insulin-like growth factor I is thought to activate intrinsic tyrosine kinase (58) in luteal cells, but the downstream effectors of activation of tyrosine kinase that lead to increased secretion of progesterone are not clearly understood. Binding of IGF-I stimulates phosphorylation of insulin receptor substrate 1 and increases the activity of phosphoinositide 3-kinase. This kinase produces the novel phosphoinositide phosphatidylinositol 3-phosphate, which may act as a second messenger (58). This substance appears to influence modification of the cytoskeleton and may be involved in prevention of cellular death. Therefore, IGF-I may acutely stimulate secretion of progesterone through modification of the cytoskeleton, while inhibition of cellular death may help maintain luteal weight (74, 204, 274). Where examined, both IGF-I and GH receptors were localized to LLC; thus GH and IGF-I may be important to maintain the high basal levels of progesterone secreted from these cells.

3. Acute stimulatory effects of prostaglandins on progesterone secretion

It has long been postulated that prostaglandins of the E and I series may be important for normal luteal function (137, 228, 229). These prostaglandins are produced in higher amounts in the early luteal phase than the late luteal phase and thus are proposed to play a role in luteal development (229). Strong evidence to support a role for PGI₂ in luteal development was pro-
vided by an experiment by Homeida and El-Eknah (156), who demonstrated that oxytocin antagonist-induced delays in luteal development were due to suppression of PGI2 synthesis. Addition of PGI2 to luteal tissue from cows, ewes, and humans also increased secretion of progesterone (5, 33, 103, 106, 228). In humans, treatment of luteal cells with PGI2 was shown to increase cAMP accumulation, suggesting that PGI2 may increase secretion of progesterone through activation of PKA. However, in the ewe, both small and LLC responded to PGI2 with increases in progesterone secretion (103). Binding sites for PGI2 reside on both small and LLC (61, 62), which makes elevation of cAMP levels an unlikely mechanism for PGI2-induced increases in progesterone production in large cells due to the inability of cAMP to increase progesterone production in this cell type (103, 161). Thus the intracellular mechanism by which PGI2 increases secretion of progesterone is unclear.

Prostaglandin E2 also has been shown to increase progesterone production from luteal cells in cows and sheep (5, 103, 106, 328). In sheep, the majority of the high-affinity receptors for PGE2 are on LLC (105), and only LLC respond to PGE2 with increased secretion of progesterone (103). In early studies with bovine luteal tissue, addition of PGE2 increased levels of cAMP and presumably activated PKA (212). However, in purified preparations of ovine LLC, addition of PGE2 did not result in increased concentrations of cAMP or activation of adenylyl cyclase (103). In addition, pharmacological activation of adenylyl cyclase with forskolin did not increase secretion of progesterone from ovine LLC (103, 161). Thus it seems likely that binding of PGE2 to its receptor in LLC also activates some unknown effector system to increase progesterone synthesis. There are multiple types of receptors for PGE2 that are linked to different second messenger systems (244).

F. Mechanisms by Which Luteotrophic Hormones Increase Secretion of Progesterone From Luteal Cells

In SLC of most species and LLC of rats and nonhuman primates, luteotropic hormones, such as LH and PGI2, dramatically increase progesterone synthesis by activation of PKA. Activation of PKA likely stimulates progesterone by increased transport of cholesterol to the P450scc enzyme complex (386). In contrast, in LLC of ewes, pigs, cows, and humans, increased synthesis of progesterone in response to luteotropins such as PGI2, PGE2, GH, and IGF-I is not mediated through increased activation of PKA. This may be because PKA in these cells is constitutively activated, as suggested by their higher basal rate of progesterone production and higher concentration of cAMP (161). Stimulation of progesterone production in LLC appears limited, and the primary regulation of progesterone secretion in this cell type appears to be negative.

V. LUTEOLYSIS

Luteolysis is defined as lysis or structural demise of the corpus luteum. During normal luteolysis, two closely related events occur. First, there is loss of the capacity to synthesize and secrete progesterone (223) followed by loss of the cells that comprise the corpus luteum (187; reviewed in Pate, Ref. 276). In most mammalian species, normal luteolysis is dependent on the presence of the uterus. Hysterectomy of heifers (8, 209, 385), ewes (385), pigs (9), guinea pigs (147), and many other species results in delayed luteolysis. However, hysterectomy of primates does not result in delayed luteolysis. Prostaglandin F2α is the factor from the uterus that initiates luteolysis (138, 222) in most nonprimate species. On the basis of the effects after partial hysterectomy and vascular anastomosis studies, initiation of luteolysis by PGF2α in many species appears to be a local effect between each uterine horn and its ipsilateral ovary (75–78; reviewed in Ginther, Ref. 120). It has been postulated that PGF2α enters the ovarian artery from the utero-ovarian vein, via a countercurrent exchange mechanism (reviewed in Ref. 120). This allows PGF2α to travel to the ovarian artery without entering the pulmonary circulation where it would be enzymatically inactivated in the lungs (285).

During luteal regression, initial decreases in concentrations of progesterone in serum do not appear to be due to loss of steroidogenic luteal cells, since numbers of luteal cells do not decrease until after concentrations of progesterone in serum have decreased (39). The decreased secretion of progesterone is most likely due to decreased luteal blood flow and decreased steroidogenic capacity of individual luteal cells. On day 15 of the estrous cycle, concentrations of several mRNA, such as those encoding 3β-HSD and StAR, are highly variable between animals due to differences in timing of the initiation of luteolysis. Thus evaluation of the role played by specific mRNA in PGF2α-induced decreases in synthesis of progesterone has been performed following use of exogenous PGF2α to ensure precise timing of the onset of luteolysis.

One of the intriguing questions about luteolysis is, What is the signal that initiates the release of PGF2α? It has been proposed that estradiol from the developing preovulatory follicle triggers the release of hypophysial oxytocin (221), which in turn stimulates release of a small quantity of uterine PGF2α (97). Prostaglandin F2α then initiates a positive-feedback loop involving release of additional uterine oxytocin and PGF2α of both luteal (369) and...
uterine origin (reviewed in Silvia et al., Ref. 336). Oxytocin (157, 237, 377) stimulates synthesis and secretion of PGF$_{2\alpha}$ from the uterus of guinea pigs (200), heifers (197, 308), mares (127), sows (186), and ewes (376). It has recently been proposed that release of luteal PGF$_{2\alpha}$ amplifies the luteolytic signal in an autocrine or paracrine manner (263, 369, 370).

A. Blood Flow and Vascular Changes

Many of the physiological, biochemical, and cellular facets of PGF$_{2\alpha}$ action on the corpus luteum have been characterized (Fig. 7). Prostaglandin F$_{2\alpha}$ reduces blood flow to the corpus luteum and, thus, may cause luteolysis by depriving the gland of nutrients, substrates for steroidogenesis, and luteotropic support (284). Administration of PGF$_{2\alpha}$ to ewes reduced blood flow to the corpus luteum in parallel to decreased secretion of progesterone (246, 251). Because endothelial cells express receptors for PGF$_{2\alpha}$ (210), PGF$_{2\alpha}$ likely acts directly on this cell population. Prostaglandin F$_{2\alpha}$ causes degeneration of luteal endothelial cells (267, 321), resulting in a marked reduction in capillary density (20, 39, 246), thereby reducing blood flow to the luteal parenchyma. It appears that even relatively low levels of PGF$_{2\alpha}$ can induce apoptosis in luteal capillary endothelial cells (Juengel and Niswender, unpublished data).

Recently, endothelin-1 has been implicated as a possible mediator of the effects of PGF$_{2\alpha}$ on luteal blood flow (121, 122). Prostaglandin F$_{2\alpha}$ stimulates endothelial cells of corpora lutea to produce endothelin-1 in vitro (122) and in vivo (262). In addition to its potent vasoconstrictive activity (reviewed in Huggins et al., Ref. 162), endothelin-1 also inhibits the steroidogenic activity of enriched populations of steroidogenic luteal cells (122). In addition, endothelin-1 may reduce blood flow during early luteolysis by causing arteriole constriction (262), and the resulting hypoxia may cause release of additional endothelin-1 (296). The antiluteolytic properties of PGE$_2$ (292) may, in part, be manifest in its ability to attenuate the vasoconstrictive actions of endothelin-1 (335).

B. Morphological Changes

Prostaglandin F$_{2\alpha}$ elicits a remarkable series of morphological changes. The proportion of steroidogenic luteal cells occupying the corpus luteum decreases within 24 h in ewes treated with PGF$_{2\alpha}$ in the midluteal phase (39). The number of LLC that can be recovered from enzymatically dispersed corpora lutea decreases, and this decrease precedes a decrease in the number of small steroidogenic luteal cells (39). A reduction in the size of LLC also occurs at this time (39, 118).

In steroidogenic luteal cells (small and large, collectively), morphological changes do not become evident until 24–36 h after exposure to PGF$_{2\alpha}$ (321), although the steroidogenic capacity of the cells is markedly reduced by this time. Interestingly, endothelial cells in capillaries of corpora lutea from ewes treated with PGF$_{2\alpha}$ are the first population of cells to exhibit dramatic morphological changes (321) that are indicative of apoptosis (185). It is suspected that degeneration of endothelial cells is a direct effect of PGF$_{2\alpha}$, but this has not been proven.
C. Intracellular Signaling

Prostaglandin F$_2\alpha$ acts by binding to specific receptors localized to large steroioidogenic luteal cells (Fig. 4; Refs. 105, 180). These receptors belong to the seven-transmembrane family of G protein-coupled receptors (2, 128, 316, 356). Upon binding to high-affinity receptors, PGF$_{2\alpha}$ induces activation of membrane-bound PLC (35) via a stimulatory G protein (231). Phospholipase C catalyzes the hydrolysis of phosphatidylinositol 4,5-bisphosphate to inositol 1,4,5-triphosphate (IP$_3$) (73) and 1,2-diacylglycerol (DAG) (35). Increased cytosolic concentrations of IP$_3$ result in the release of free Ca$^{2+}$ from the smooth endoplasmic reticulum to the cytoplasmic compartment (35). Increased free Ca$^{2+}$ and DAG (localized to the plasma membrane) stimulate the catalytic activity of Ca$^{2+}$-dependent protein kinase (PKC; also localized to the plasma membrane; Ref. 247).

Protein kinase C refers to a family of serine/threonine protein kinases (248) that exists in 11 isoforms identified to date (reviewed in Quest, Ref. 294). Differences among isoforms include subcellular localization and Ca$^{2+}$ dependence (294). The α- (cytosolic) and ε-isoforms (plasma membrane) of PKC are immunochemically detectable in the bovine corpus luteum (266), whereas the δ-isoform is the predominant form in the corpus luteum of pseudopregnant rats (71). Protein kinase C-α is calcium dependent, whereas PKC-δ and PKC-ε are not calcium dependent (249). Both calcium-dependent and calcium-independent isoforms of PKC contain highly-conserved, cysteine-rich domains involved in binding of DAG and pharmacological activators of PKC such as PMA (32). Translocation of PKC-α from the cytosol to the nucleus is stimulated by phorbol esters in some cell types (88, 199, 363). The array of isoforms, their subcellular distribution, and their roles in the regulation of the corpus luteum have received little attention to date.

Protein kinase C is believed to mediate many of the antisteroidogenic actions of PGF$_{2\alpha}$ in LLC (Fig. 4; Refs. 223, 387, 388). In support of this view, purified LLC cultured with PGF$_{2\alpha}$ exhibit an influx of extracellular Ca$^{2+}$ to the cytosolic compartment similar to the influx observed in response to A-23187 (a Ca$^{2+}$ ionophore; Ref. 389). Prostaglandin F$_{2\alpha}$-induced accumulation of microsomal-derived and extracellularly derived Ca$^{2+}$ in the cytosolic compartment enhances the catalytic activity of PKC. Activation of PKC in LLC is believed to result in posttranslational modification of cellular proteins involved in steroidogenesis (223, 387), cholesterol availability (29), and maintenance of the extracellular matrix (392; reviewed in Lum and Malik, Ref. 206). Although there is pharmacological activation of PKC in the corpus luteum under conditions that dramatically decrease steroidogenesis, such activation does not cause apoptosis (223). Activation of PKC induces expression and activation of proteins involved in apoptosis in other cell types (reviewed in Schwartzman and Cidlowski, Ref. 325). It is possible that apoptosis in LLC is facilitated by PKC activation.

D. Luteal PGF$_{2\alpha}$

Prostaglandin F$_{2\alpha}$ can be synthesized by corpora lutea of women (279, 333, 358; reviewed in Mitchell et al., Ref. 230), sows (133), ewes (303, 369), cows (275), and rodents (263). During the late luteal phase of the estrous cycle, PGF$_{2\alpha}$ from peripheral sources can stimulate synthesis of PGF$_{2\alpha}$ in corpora lutea of ewes (369).

In contrast to ruminants, luteolysis in primates is not mediated by uterine PGF$_{2\alpha}$ (31). However, it has been proposed that PGF$_{2\alpha}$ produced locally in the corpus luteum acts via a paracrine and/or autocrine mechanism to induce luteolysis (19). Whether PGF$_{2\alpha}$ is responsible for luteolysis and its source remain controversial. Prostaglandins F$_{2\alpha}$ and E$_{2}$ are synthesized using membrane phospholipids as substrate in a three-step series of reactions termed the cyclooxygenase pathway. Phospholipases A$_{2}$ and C, localized to the plasma membrane, hydrolyze membrane phospholipids, liberating arachidonic acid that can be utilized as substrate for PGF$_{2\alpha}$ synthesis (184). Cyclooxygenase (prostaglandin G/H synthase) catalyzes the rate-limiting step in prostaglandin biosynthesis, which is conversion of arachidonic acid to PGH$_{2}$ (reviewed in Dewitt and Smith, Ref. 83). Finally, PGH$_{2}$ is rapidly converted to PGF$_{2\alpha}$ by prostaglandin F synthase (380).

Phospholipases A$_{2}$ and C exhibit increased enzymatic activity in the presence of elevated intracellular free Ca$^{2+}$ (1, 109). The ovine corpus luteum expresses cyclooxygenase in response to PGF$_{2\alpha}$ (369). However, PGF$_{2\alpha}$ down-regulates mRNA encoding cyclooxygenase in the bovine corpus luteum early in the estrous cycle (370). This period coincides with a time when PGF$_{2\alpha}$ cannot cause luteolysis, suggesting local synthesis of PGF$_{2\alpha}$ may be important in this process. These observations support the notion that PGF$_{2\alpha}$ can autoregulate its synthesis by stimulating the liberation of arachidonic acid by hydrolysis of membrane phospholipids as a result of 1) PGF$_{2\alpha}$ receptor-coupled G protein activation of phospholipase C, and conversion of arachidonic acid to PGH$_{2}$, and 2) increased cytosolic Ca$^{2+}$. Availability of arachidonic acid and cyclooxygenase activity, which determine a cell’s capacity to synthesize prostaglandins, are both increased in the corpus luteum in response to PGF$_{2\alpha}$.

E. PGF$_{2\alpha}$ Inhibition of Progesterone Synthesis

Prostaglandin F$_{2\alpha}$ decreases luteal synthesis of progesterone in cows, ewes, sows, monkeys, humans, and pseudopregnant/pregnant rats and rabbits in vivo (reviewed in Niswender and Nett, Ref. 254). However, the
negative effects of PGF$_{2a}$ on luteal progesterone secretion in vitro have been more difficult to demonstrate and require appropriate culture conditions. If luteal cells from the mid to late luteal phase are supplied with lipoproteins as a source of cholesterol which enhances the secretion of progesterone, treatment with PGF$_{2a}$ results in decreased synthesis and secretion of progesterone (104, 278, 288, 388, 397). There are likely multiple mechanisms by which PGF$_{2a}$ decreases synthesis of progesterone; however, because PGF$_{2a}$ decreases progesterone secretion from purified preparations of ovine and bovine LLC (388), it is clear that PGF$_{2a}$ directly influences these cells (Fig. 5). Prostaglandin F$_{2a}$ could decrease progesterone synthesis by a number of intracellular mechanisms, including 1) downregulation of receptors for luteotropic hormones, 2) decreased cellular uptake of cholesterol, 3) decreased transport of cholesterol through the cell and/or across the mitochondrial membranes, and 4) decreased activity of the steroidogenic enzymes required for biosynthesis of progesterone.

1. Effects of PGF$_{2a}$ on receptors for luteotropic hormones

Because normal rates of synthesis of progesterone are dependent on luteotropic hormones, PGF$_{2a}$ could reduce responsiveness of luteal tissue to these hormones. Treatment of ewes with PGF$_{2a}$ causes a rapid decrease in mRNA encoding the receptor for LH (134, 341). This decrease in concentrations of mRNA for LH-R appears to precede a decrease in binding of LH to luteal membranes (86). However, the decrease in receptors for LH occurred after concentration of progesterone in serum had already decreased in both ewes (86) and cows (346). In addition, PGF$_{2a}$ did not affect concentrations of mRNA encoding GH-R mRNA before a decrease in concentrations of progesterone (178) and binding of IGF-I to luteal tissue after treatment with PGF$_{2a}$ was not decreased (281). Thus downregulation of receptors for luteotropic hormones does not appear to be a mechanism by which PGF$_{2a}$ decreases secretion of progesterone from the corpus luteum. However, PGF$_{2a}$ may interfere with the ability of LH to activate PKA as activity of adenylate cyclase decreases after administration of PGF$_{2a}$. One mechanism by which PGF$_{2a}$ may decrease activity of PKA is through increased degradation of cAMP by increased activity of phosphodiesterase (4, 117). In addition, if PKA is constitutively activated in LLC as has been suggested, the ability of PGF$_{2a}$ to suppress activity of PKA may lead to decreased secretion of progesterone from LLC.

2. Effect of PGF$_{2a}$ on uptake of lipoprotein and release of cholesterol

If the antisteroidogenic effects of PGF$_{2a}$ were mediated through regulation of uptake of lipoproteins or storage and release of cholesterol, one would expect a decrease in lipoprotein receptors and/or activity of cholesterol esterase. In vivo, PGF$_{2a}$ decreased luteal concentrations of mRNA encoding LDL-R (312, 360) but increased mRNA for HDL-BP in ewes (360). In addition, treatment of rats with PGF$_{2a}$ did not decrease HDL uptake (295). Because ovine luteal cells preferentially utilize HDL for a cholesterol source (387) and suppression of LDL-R mRNA did not decrease concentrations of progesterone in serum (T. Tandeski, J. Juengel, and G. Niswender, unpublished observations), it does not seem likely that decreased uptake of lipoprotein occurs after PGF$_{2a}$ treatment in vivo. A lack of effect of PGF$_{2a}$ on lipoprotein uptake has also been shown in experiments utilizing bovine and ovine luteal cells (131, 386). Thus PGF$_{2a}$ does not appear to regulate the uptake of cholesterol from extracellular sources. Likewise, the activity of cholesterol esterase was not affected by PGF$_{2a}$ treatment of ovine LLC (386).

3. Effects of PGF$_{2a}$ on transport of cholesterol

Transport of cholesterol within luteal cells can be broken down into two phases: transport of cholesterol through the cytosol to the outer mitochondrial membrane and transport of cholesterol from the outer to the inner membrane where the enzymes required for side-chain cleavage reside (Fig. 5). Transport of cholesterol in the cytoplasm appears to involve interactions between sterol binding proteins and the cytoskeleton. One protein thought to be important for transport of cholesterol is sterol carrier protein (SCP-2). Treatment of rats with PGF$_{2a}$ decreases the amount of SCP-2 (224), which may reduce the transport of cholesterol to the mitochondria. Disruption of the cytoskeleton is known to decrease secretion of progesterone from luteal cells of a variety of species (320, 334, 340, 384). In ewes, treatment with PGF$_{2a}$ rapidly and dramatically reduces the number of luteal cells staining positive for tubulin, the major component of microtubular fibers (241). The disappearance of tubulin occurs before decreased luteal concentrations of progesterone, indicating that disruption of the cytoskeleton precedes decreased synthesis of progesterone. However, it is not clear whether disruption of the microtubule network prevents transport of cholesterol to mitochondria or disturbs other aspects of luteal steroidogenesis. In cell cultures, disruption of either microtubules or microfilaments either inhibits (320, 334, 384) or stimulates steroidogenesis (54, 55, 298). It was noted by Smith and Sridaran (340) that disruption of the cytoskeleton for a short time inhibited steroidogenesis, whereas disruption of the cytoskeleton for a longer time stimulated steroidogenesis. In ovine luteal cells, addition of cytochalasins decreased secretion of progesterone (320, 334). Thus
PGF$_{2\alpha}$-induced disruption of the cytoskeleton may impair synthesis of progesterone early during luteolysis.

Prostaglandin F$_{2\alpha}$ also appears to inhibit transport of cholesterol across the mitochondrial membranes in vitro (131, 386). Two systems have been identified that appear to be important for transport of cholesterol across the mitochondrial membrane to P-450$_{scc}$ in other steroidogenic tissues (Fig. 6). Steroidogenic acute regulatory protein (reviewed in Stocco and Clark, Ref. 352) and the PBR/endozepine system (271) likely interact to facilitate cholesterol transport. However, little is known about the PBR/endozepine system in luteal cells. Concentrations of mRNA encoding StAR and StAR protein were highly correlated in bovine luteal tissues collected at different stages of the estrous cycle (282). Treatment of ewes or cows with PGF$_{2\alpha}$ dramatically reduces concentrations of mRNA encoding StAR (177, 282), which is followed by a decline in StAR production (282). The reduced concentrations of STAR mRNA after PGF$_{2\alpha}$ would be expected to result in an immediate reduction in StAR protein, since its half-life is thought to be 3–5 min (92, 353). This may lead to reduced transport of cholesterol across the mitochondrial membranes. Indeed, luteal cells can maintain a high rate of progesterone secretion in the face of PGF$_{2\alpha}$ if supplied with analogs of cholesterol that freely diffuse through membranes (131, 386). Thus downregulation of transport of cholesterol across the mitochondrial membranes appears to be a primary point of negative regulation of progesterone synthesis by PGF$_{2\alpha}$. It is also interesting that there are multiple potential PKC phosphorylation sites in ovine StAR that could provide a mechanism for direct regulation of cholesterol transport by phosphorylation of this protein (Fig. 6; Juengel and Niswender, unpublished observations).

4. Effects of PGF$_{2\alpha}$ on conversion of cholesterol to progesterone

For years, conversion of cholesterol to pregnenolone by the P-450$_{scc}$ complex was thought to be the hormonally regulated step in steroidogenesis. However, doses of PGF$_{2\alpha}$ that decrease progesterone secretion do not decrease mRNA encoding P-450$_{scc}$ protein, or activity of the P-450$_{scc}$ enzyme complex (30, 131, 223, 311, 364, 386). In contrast to the lack of effect of PGF$_{2\alpha}$ on P-450$_{scc}$, PGF$_{2\alpha}$ rapidly decreases 3β-HSD mRNA (143, 364). However, during the initial 24 h after treatment with PGF$_{2\alpha}$, neither amount of 3β-HSD protein nor enzyme activity was affected by treatment with PGF$_{2\alpha}$, although mRNA encoding 3β-HSD was greatly reduced in these same tissues (175, 176, 311). Thus PGF$_{2\alpha}$ does not appear to decrease progesterone secretion through regulation of P-450$_{scc}$ or 3β-HSD activity.

F. Negative Regulation of Progesterone in Small Luteal Cells

In vivo, the mechanisms by which administration of PGF$_{2\alpha}$ causes decreased progesterone secretion in SLC in ewes is somewhat controversial, since the presence of receptors for PGF$_{2\alpha}$ on these cells is controversial (23, 105). High-affinity receptors for PGF$_{2\alpha}$ are not found on ovine SLC (23, 105), and treatment of SLC with PGF$_{2\alpha}$ does not affect secretion of progesterone (386, 390). However, activation of PKC inhibits PKA-stimulated progesterone production by SLC (386). Activation of PKC in these cells did not affect uptake of lipoproteins, P-450$_{scc}$ activity, or 3β-HSD activity. Thus it was concluded that the mechanism for decreased progesterone secretion in small cells was due to reduced transport of cholesterol through the cell and/or across the mitochondrial membranes (386). The identity of the hormone that activates PKC in small cells is not known. However, oxytocin receptors are present on porcine SLC (397); oxytocin decreases secretion of progesterone from bovine, porcine, and human luteal tissue/cells (34, 287, 397); PGF$_{2\alpha}$ causes release of luteal oxytocin (108); and oxytocin binding to its receptor causes an increase in inositol phosphate turnover (107), which would be expected to lead to an increase in PKC activity. However, release of luteal oxytocin with norepinephrine did not decrease progesterone secretion from bovine luteal tissue (191). In addition, oxytocin-induced downregulation of progesterone secretion from human corpora lutea was found to be attributable to synthesis of PGF$_{2\alpha}$ (34). Thus negative regulation of synthesis of progesterone from SLC by oxytocin or other factors remains unresolved.

G. Immune-Mediated Events

There is compelling evidence for a critical role of the immune system in the process of luteolysis. Splenectomy in rats results in elevated concentrations of progesterone in serum and delayed ovulation during the time of normal luteal regression, and this effect is reversed by injection of splenocytes (218). In addition, immunosuppressive doses of dexamethasone delay natural but not PGF$_{2\alpha}$-induced luteolysis in rats (379). Glucocorticoids such as dexamethasone inhibit leukocyte accumulation in injured tissues and abrogate the synthesis and action of many cytokines (146).

Leukocytes infiltrate the corpus luteum during luteolysis (44; reviewed in Murdoch et al., Ref. 243). Eosinophils, like macrophages, accumulate in the regressing corpus luteum before the decline in serum levels of progesterone in response to an uncharacterized chemotactic factor (240). Eosinophils, however, are not requisite for PGF$_{2\alpha}$-induced luteal regression to occur (242). Treat-
ment of ewes in the midluteal phase with PGF$_{2\alpha}$ (145, 368) or PMA (368) induces luteal expression of mRNA encoding monocyte chemoattractant protein-1 in corpora lutea. Macrophages infiltrate the parenchyma and blood vessels of the porcine corpus luteum during PGF$_{2\alpha}$-induced luteolysis before any precipitous decline in progesterone secretion (149). The primary role of macrophages during luteal regression appears to be phagocytosis of degenerative luteal cells (3, 48, 268, 280) and degradation of the extracellular matrix (272, 372). Macrophages perform three functions during the early stages of luteolysis: (1) phagocytosis of degenerative luteal cells, (2) cytokine-mediated inhibition of steroidogenesis, and (3) stimulation of PGF$_{2\alpha}$ secretion by the corpus luteum.

During luteolysis, T lymphocytes infiltrate the corpus luteum and secrete interferon-$\gamma$ (IFN-$\gamma$), which stimulates presentation of major histocompatibility complex antigens on the surface of luteal cells (95). Interleukin-1 (IL-1) produced by macrophages, fibroblasts, and endothelial cells (268) stimulates production of PGF$_{2\alpha}$ by cultured bovine luteal cells (258). In addition, tumor necrosis factor-$\alpha$ (TNF-$\alpha$), produced by macrophages, inhibits basal progesterone secretion and stimulates PGF$_{2\alpha}$ secretion (96). Production of bioactive TNF-$\alpha$ begins after the loss of progesterone synthesis in the ovine (171) and bovine corpus luteum (327), but TNF-$\alpha$ could serve to complement the luteolytic activity of uterine PGF$_{2\alpha}$ during luteolysis by stimulating synthesis of luteal PGF$_{2\alpha}$. Consistent with this notion, TNF-$\alpha$ and IFN-$\gamma$ synergistically enhance synthesis and secretion of PGF$_{2\alpha}$ by cultured bovine luteal cells (96). Thus attraction and infiltration of leukocytes into the luteal parenchyma results in increased local production of cytokines (particularly IL-1, TNF-$\alpha$, and IFN-$\gamma$) that ultimately stimulate luteal PGF$_{2\alpha}$ synthesis and activate macrophages. In summary, immune cells and cytokines appear to play a role in luteolysis by regulating PGF$_{2\alpha}$ synthesis, steroidogenesis, and phagocytosis.

H. Apoptosis

Apoptosis (215; reviewed in Schwartzman and Cidlowski, Ref. 325) is an active, energy-dependent process by which nonessential populations of cells delete themselves from a tissue (185). Several fundamental biochemical and morphological changes are characteristics of apoptotic cells including nuclear fragmentation, appearance of membrane-bound vesicles of cytoplasmic contents, laddering of genomic DNA, and changes in gene expression. The involution of many endocrine glands after removal of tropic hormone support or activation by a negative stimulus is achieved by apoptosis. Granulosa cells, deprived of FSH, undergo apoptosis during follicular atresia (163, 365). Epithelial cells of the human endometrium experience apoptosis when progesterone and estradiol support declines (158). Prostaglandin F$_{2\alpha}$ promotes apoptosis in cells comprising the corpus luteum (321).

The first morphological evidence that a cell is apoptotic is the appearance of nuclear fragments containing degenerate chromatin (321), cell shrinkage, and appearance of membrane-bound cytoplasmic fragments (185). These cell fragments, or apoptotic bodies, are targets for the phagocytic cells of the immune system. Macrophages augment the apoptotic process in populations of luteal cells by phagocytosing membrane-enclosed fragments of those cells (118).

Another characteristic feature of apoptosis is internucleosomal cleavage of genomic DNA into 185-bp fragments (oligonucleosomes; Ref. 13). This characteristic DNA fragmentation, seen as a ladder pattern on agarose gels (13), is the result of activation of Ca$^{2+}$-dependent endonucleases (395). Evidence for a role of PGF$_{2\alpha}$ in apoptosis of luteal cells is the appearance of oligonucleosomes in response to PGF$_{2\alpha}$ in cattle (173, 399), sheep (223, 315), humans (329), rats (217), and pigs (21).

Recently, considerable interest has developed in the roles of bcl-2 family members in regulating apoptosis (214, 301; reviewed in Korsmeyer, Ref. 190). The first protein identified that regulates apoptosis was bcl-2 (189). Membrane-associated bcl-2 prevents cell death by regulating the maintenance of Ca$^{2+}$ homeostatic mechanisms (22), attenuating oxidative stress (152), and interacting with ras (growth factor signal transduction; Ref. 101) and bax (190). Bax (bcl-2-associated-gene-x) promotes apoptosis by binding to, sequestering, and antagonizing the cell survival functions of bcl-2 (264) and directly promoting apoptosis (190). The ratio of bcl-2 and bax within a cell is related to that cell’s potential to become apoptotic. The nuclear protein p53 functions to stimulate apoptotic cell death by increasing transcription rates of the bax gene (233, 234) while repressing transcription of bcl-2 (232, 233). Two homologs of bcl-2 have been identified, bcl-xL and bcl-xS; both proteins are the products of alternatively spliced transcripts from the bcl-x gene (36). Bcl-xL functions as a repressor of apoptosis (similar to bcl-2), whereas bcl-xS mimics the actions of bax (36).

During luteolysis in cattle, mRNA encoding bax is elevated while mRNA encoding bcl-2 remains unchanged (313), resulting in an increased ratio of bax to bcl-2, an event consistent with bax-mediated apoptosis. It remains unclear whether increases in bax mRNA are p53 mediated. Levels of mRNA encoding p53 do not increase during luteolysis (313, 366); however, p53 activity may be regulated primarily at the posttranslational level (398).

I. Oxidative Stress

Reactive oxygen compounds are integrally involved in luteolysis and apoptosis (52, 307). Superoxide
anion radicals, hydroxyl radicals, and hydrogen peroxide (H₂O₂) are the primary reactive oxygen species generated in steroidogenic cells (159, 160). An appreciable amount of oxidative stress experienced during luteolysis is possibly produced by macrophages (338) within the regressing corpus luteum. The potentially toxic effects of these substances are attenuated by antioxidant vitamins (ascorbate, α-tocopherol; Refs. 207), enzymes [catalase, superoxide dismutase (SOD), isozymes, and glutathione peroxidase (reviewed in Rueda et al., Ref. 314)], and, to a lesser extent, stabilization of radicals by transfer of unpaired electrons to polyunsaturated membrane lipids (16). Superoxide dismutase enzymes catalyze the conversion of superoxide anion radical to the more stable H₂O₂, which is then converted to H₂O by catalase or glutathione peroxidase (reviewed in Yu, Ref. 396). Antioxidant vitamins stabilize free radicals by forming resonance structures upon acceptance of unpaired electrons (326).

Ascorbate concentrations in the ovine corpus luteum are 5- to 38-fold greater than in other tissues (324) but depleted concurrent with luteal regression (91) and lipid peroxidation (16, 319). Consistent with the concept of decreased cellular protection against oxidative stress, levels of mRNA encoding secreted and mitochondrial (Mn-dependent)-SOD and catalase are decreased in regressing bovine corpora lutea (314).

VI. MATERNAL RECOGNITION OF PREGNANCY

A. General Considerations

The requirement for progesterone during gestation exceeds the length of the normal estrous cycle in most mammals. In most species, the pregnant female quickly recognizes that a conceptus is present (embryo and associated membranes) (57, 148, 227, 238, 265). Maternal recognition of pregnancy involves biochemical communication between the conceptus and its mother to provide uninterrupted synthesis and release of progesterone. Extension of luteal function beyond the length of the estrous cycle is the first evidence that maternal recognition of pregnancy has occurred (153, 331). Although the mechanisms by which progesterone synthesis is maintained vary between species, only a few general types of signals are used, and timing of the signal is critical. In general, the conceptus secretes factors that either prevent the secretion or luteolytic actions of PGF₂α or that are directly luteotropic. The detailed mechanisms involved in maternal recognition of pregnancy have recently been reviewed (309).

B. Maternal Recognition of Pregnancy in Ruminants

In domestic ruminants, the period of time that the pregnant female is dependent on luteal progesterone for maintenance of pregnancy varies. In sheep, the shift from dependence on luteal to placental progesterone occurs after ~45 days of gestation (56, 80). In cattle, the embryo/fetus is dependent on luteal progesterone for 200 days of gestation (63, 93).

Early observations that prevention of luteolysis is extended only to the corpus luteum, which is ipsilateral to the gravid uterine horn (235, 236, 252), provided strong evidence that the embryonic signal acts locally rather than systematically. In sheep, the signal must be present at adequate concentrations on days 12–15 (124, 373) and in cattle on days 14–17 (257). In these species, the conceptus produces a unique signal, IFN-γ, that extends luteal function beyond the length of the estrous cycle by blocking the synthesis of PGF₂α and preventing luteolysis. Interferon-γ is produced in pregnant ewes from days 11 to 23 (10, 98, 123), with peak production occurring between days 14 and 16 (125, 139) after estrus. In pregnant cows, secretion of IFN-γ peaks between day 17 and 18 after estrus (25, 150).

Interferon-γ is produced by the trophoderm of the conceptus (28) and extends the life span of the corpus luteum by indirect mechanisms. Interferon-γ attenuates secretion of uterine PGF₂α in cattle (72, 126, 226) and sheep (317) by suppressing the transcription of genes that encode receptors for estradiol and oxytocin (344). The reduction of estradiol receptor numbers further suppresses oxytocin receptor gene expression by blocking estradiol-mediated upregulation of this gene. Progesterone provides an additional negative effect on estradiol and oxytocin receptors.

It has been demonstrated that the primary site of action for IFN-γ is the luminal and superficial glandular epithelium (345), whereas the primary site of action of progesterone on oxytocin and estradiol receptors is the deep glandular epithelium during pregnancy (345). A combination of IFN-γ and progesterone prevents oxytocin-mediated PGF₂α release from the uterus and subsequent luteolysis.

The ruminant conceptus may also utilize additional mechanisms for extension of the life span of corpus luteum. A second mechanism involves the secretion, by the day 15 sheep conceptus, of a protein that blocks the effects of PGF₂α on the LLC by unknown mechanisms (391). This protein does not bind or metabolize PGF₂α, but rather blocks the effect via some intracellular mechanism(s). Thus the ruminant conceptus ensures that its requirement for luteal progesterone is met by preventing uterine secretion of PGF₂α or preventing the actions of PGF₂α at the luteal cell level. Because luteal progesterone
is essential for embryonic survival and development, it is not surprising that redundant protective pathways are present to ensure survival of the corpus luteum.

C. Maternal Recognition of Pregnancy in Rodents

Stimulation of the cervix during copulation or mating to a sterile male causes activation of a neuroendocrine reflex arc that results in a dramatic release of prolactin, LH, and FSH on the morning of estrus (347). There is also a biphasic release of prolactin from the pituitary that continues for 8–10 days. The release of prolactin extends the function of the corpus luteum for 10–12 days, and the female enters a period in which she is considered to be pseudopregnant if embryos are not present in the uterus (342). If the female is pregnant, the biphasic release of hypophysial prolactin also continues for 8 days. In pseudopregnant rats, on day 8 the diurnal release of prolactin ends, but the nocturnal release continues for 2 days (342) and then decreases until day 12 when a new estrous cycle begins.

In pregnant rats, the timing of the loss of the diurnal release of hypophysial prolactin corresponds to the day at which placental lactogen produced by the embryo becomes detectable in serum (219, 330). The maximum concentration of placental lactogen is reached on day 10 (330), the day at which the nocturnal release of hypophysial prolactin terminates. It is from day 10 to the end of gestation that the corpus luteum is supported by placental luteotrophin rather than a hypophysial luteotrophin (196). There is also an increase in androgen production, which serves as substrate for estradiol synthesis in the placenta. The rodent corpus luteum expresses prolactin (90) and estradiol receptors and responds to these hormones with increased production of progesterone and cell hypertrophy. Prolactin appears to downregulate 20α-hydroxysteroid dehydrogenase gene expression (400), but the mechanism is not yet understood. Prolactin also causes activation of elongation factor-2, an essential component of protein synthesis, by dephosphorylation.

D. Maternal Recognition of Pregnancy in Primates and Equids

The mechanisms involved in maternal recognition of pregnancy in primates and equids are unique and involve production of a signal from the conceptus, chorionic gonadotrophin (CG). In these species, as in most others, the life span of the corpus luteum during the normal reproductive cycle is not adequate to provide progesterone for maintenance of pregnancy until the shift to placental progesterone can occur. The prime conceptus by days 8–12 of pregnancy begins to produce CG, which rescues the corpus luteum by providing a direct luteotropic signal until the shift to the placenta as the source of progesterone can occur. Chorionic gonadotrophin is structurally and biologically similar to LH and directly stimulates the primate corpus luteum to secrete progesterone.

In equids, the mechanisms responsible for maternal recognition of pregnancy are more complex than those in primates. Production of CG is not detectable until approximately day 35 of pregnancy. It appears that the equine conceptus alters the ratio of PGE2 versus PGF2α in the uterine vein and that PGE2 stimulates continued luteal function until day 35 of pregnancy.

The half-life of hCG in blood is considerably longer than that of LH because of the increased number of amino acids and the increased carbohydrate content of the subunit of hCG, which results in enhanced biological activity. In equids, the CG promotes follicular growth and ovulation and formation of accessory corpora lutea which can number up to 70 in a single mare. The accessory corpora lutea are then responsible for continued secretion of the progesterone required to maintain pregnancy until approximately day 160 of pregnancy, at which time placental secretion of progesterone maintains pregnancy.

In summary, the mechanisms by which the progesterone needed for establishment and maintenance of pregnancy is regulated by the mammalian conceptus vary greatly. However, in all cases, adequate luteal progesterone is secreted to allow maintenance of pregnancy until a conceptus-derived source of progesterone, the placenta, can produce adequate amounts of this steroid to complete the gestation interval and result in a healthy offspring.

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