AN ABSTRACT OF THE THESIS OF

Carrie Ann Cosola-Smith for the degree of *Master of Science* in *Animal Science* presented on *April 6, 1989.*

Title: *Bovine Luteal Oxytocin Synthesis and Secretion in vitro: Effects of Phorbol Ester, Calcium Ionophore and Indomethacin*

Abstract approved: Redacted for privacy

Dr. Fredrick Stormshak

Experiments were conducted to examine the effects of a tumor-promoting phorbol ester, a calcium ionophore and indomethacin on bovine luteal oxytocin (OT) synthesis and secretion and progesterone secretion. Six 18-month-old crossbred beef heifers in Exp. 1 and five similar heifers in Exp. 2 were observed twice daily for estrus using vasectomized bulls. After at least two consecutive estrous cycles of normal duration (18-23 days), corpora lutea surgically removed on day 8 of the estrous cycle (day of estrus = day 0 of the cycle) were sliced and incubated for 2 h with 0.81nM 12-*O*-tetradecanoylphorbol 13-acetate (TPA), 1.62 nM TPA, 100μM indomethacin, 1.62 nM TPA and 100 μM indomethacin or 0.3 μM calcium ionophore A23187. Both levels of TPA increased OT secretion ($ng \cdot g^{-1} \cdot 2h^{-1}$; control, 407.1; 0.81 nM TPA, 494.7; 1.62 nM TPA, 528.1; common SE = 21.2; $P<0.01$) and synthesis (control, 368.5; 0.81 nM TPA, 427.6; 1.62 nM TPA, 492.1; common SE = 25.7; $P<0.02$). Although indomethacin alone did not alter basal OT release significantly, it

inhibited TPA-induced OT secretion (TPA x indomethacin interaction, $P<0.005$) and synthesis ($P<0.10$). Calcium ionophore increased OT secretion ($ng \cdot g^{-1} \cdot 2h^{-1}$; control, 248.9; A23187, 327.4; common SE = 16.0; $P<0.01$) and synthesis (control, 129.4; A23187, 165.6; common SE = 16.4; $P\approx0.09$). The phorbol ester TPA also induced progesterone secretion ($ng \cdot g^{-1} \cdot 2h^{-1}$; control, 1056.2; 0.81 nM TPA, 1333.3; common SE = 86.4; $P<0.025$). Because TPA can activate protein kinase C and A23187 increases intracellular calcium, it is concluded that these intracellular constituents may be involved in OT synthesis and release, as well as progesterone secretion.

Bovine Luteal Oxytocin Synthesis and Secretion *in vitro*: Effects of Phorbol Ester, Calcium Ionophore and Indomethacin

by

Carrie A. Cosola-Smith

A THESIS

submitted to

Oregon State University

in partial fulfillment of

the requirements for the

degree of

Master of Science

Completed April 6, 1989

Commencement June, 1990

APPROVED:

Redacted for privacy

Professor of Animal Science in charge of major

Redacted for privacy

Head of Department of Animal Science

Redacted for privacy

Dean of Graduate School

Date thesis is presented April 6, 1986

ACKNOWLEDGEMENTS

I would like to thank Dr. Fredrick Stormshak for his contributions to the successful completion of this program. He bestowed on me an appreciation of science. His guidance was invaluable.

My deepest gratitude is also extended to Dr. Fred Menino for his advice, encouragement and most importantly, for his friendship. He gave me laughter and reassurance when it was needed most. Gratitude is also extended to Dr. Michael Schimerlik and Dr. Fink for serving as members of my committee and for their valuable criticisms and advice.

Many thanks also go to Dr. Dieter Schams of the Institute of Physiology, Technical University of Munich, Freising-Weihenstephan, West Germany, for his generous donation of rabbit anti-oxytocin serum.

Thanks are also extended to Dr. Loren Appell for performing all surgeries required for this research.

I would especially like to thank Dr. Salah Abdelgadir of the Oregon Health Sciences University for his wisdom, patience and advice. This thesis would not have been remotely possible if not for his assistance. He will be sorely missed. I am also very thankful of Dr. Tony Archibong of the Oregon Regional Primate Research Center, Beaverton, Oregon, for his technical assistance and friendship.

I would also like to thank my colleagues and friends, Dr. Kitty Gardiner, Mark Grobner, Patrice and Brian Prater, Anna Cortell, Ligaya Tumbelaka and most especially Jane Susan Williams for their encouragement, support, humor and friendship.

I thank Dr. Mary Zelinski-Wooten, Oregon Regional Primate Research Center, Beaverton, Oregon, for her keen insight, vast knowledge of scientific literature and helpful input throughout conduct of this thesis.

Dr. Stephen Ford of Iowa State University, Ames, Iowa also is thanked for his helpful suggestions and for instilling in me a deep respect and appreciation for "the big picture."

Mr. Marvin Martin is greatfully acknowledged for arranging the care of experimental animals.

I would like to thank my mother Stephanie Cosola-Hanington most importantly for her love, support, strength and for always wanting better for her children than for herself. None of this would have been possible without her. I am also deeply indebted to my step-father, Mr. Richard Hanington for his humor, support and for his love.

I would also like to thank my brother and sister, Peter and Mary Cosola for their love and for "being there" even though it was three thousand miles away. I would also like to thank my East Coast Support System: Darlene Pelton, Gina Riccio, Eddie Stankewick and the Melted Americans and many others.

I would be remiss if I did not thank my furry little friends. First I would like to thank Stewart for 13 years of purrs and for staying sane after being moved from five homes in three years. I would like to thank Chelsea for taking me on many long beautiful walks and for her friendship. I would also like to thank Tristana, Cadbury, and finally Caddis for being a happy, loveable spaz.

I am eternally thankful to my husband, Gary Daniel Smith, for his love, support and for coming along and giving it all meaning and perspective. I dedicate this thesis to my parents, but I dedicate my future to him and to us.

Finally I would like to thank my Lord God, for His love, for His Son and for all of the above.

DEDICATION

I would like to dedicate this thesis in loving memory of my father, Michael A. Cosola and to my mother, Stephanie Cosola-Hanington. His memory, their love and examples have provided me with determination to succeed and to be as good a person as they are. Thank you. I love you.

TABLE OF CONTENTS

LIST OF FIGURES

BOVINE LUTEAL OXYTOCIN SYNTHESIS AND SECRETION *IN VITRO:* EFFECTS OF PHORBOL ESTER, CALCIUM IONOPHORE AND INDOMETHACIN

LITERATURE REVIEW

The importance of the role of the corpus luteum in maintaining pregnancy has been recognized since the experiments of Fraenkel at the turn of the century. Subsequently, in the 1930's the substance that the corpus luteum secretes to maintain gestation, progesterone, was isolated and characterized (Corner, 1961). It is now known that luteinizing hormone (LH) is the primary hormone involved in maintaining the corpus luteum during the estrous cycle and early pregnancy in a number of species. Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) is recognized as the major luteolysin. In the past decade it has been discovered that the corpus luteum synthesizes and secretes oxytocin. It has been suggested that oxytocin may play an important role in the paracrine control of the corpus luteum.

Because the corpus luteum is integral to the maintenance of pregnancy, especially with respect to early gestation, it is important to fully understand the function of this organ. A more complete understanding of this organ will aid in increasing the efficiency of livestock production by improving methods for estrous synchronization and by providing a better understanding of factors affecting and causing embryonic mortality. Other possible benefits of studying the corpus luteum could be improved methods of contraception for humans and overpopulated species such as rodents, coyotes and wild horses.

Investigators have examined the corpus luteum on systemic and cellular levels for many years, yet it was not until the past decade that the molecular aspects of the corpus luteum were studied. Because all previous work has yet to yield a completely clear picture of luteal formation, maintenance and regression, it seems that more investigations at the molecular level, particularly with respect to the action of oxytocin, might serve to bring the picture into focus.

The review of literature will encompass discussions of research that has been conducted to define the physiological processes by which the corpus luteum arises and the endocrinological factors that govern its maintenance and regression. Although research on the formation and function of corpora lutea in domestic animals will be emphasized, pertinent results of studies on the corpus luteum of other species will be included when appropriate.

Folliculogenesis

Corpora lutea develop from granulosa and theca cells of ovulated follicles. In order to gain an appreciation for the complexity of luteal development, as well as an understanding of the origin and functions of various luteal cell populations, it is deemed appropriate to review the process of folliculogenesis. In mammals folliculogenesis encompasses marked changes in the morphological and biochemical characteristics of the cells destined to contribute to formation of the corpus luteum.

Folliculogenesis is the process whereby primordial follicles develop into preantral, antral (secondary and tertiary) and then ovulatory (Graafian) follicles. The first crucial step in folliculogenesis, recruitment, is characterized by the development of a group of primordial follicles into follicles that contain an

antrum. Development prior to recruitment does not appear to be gonadotropin-dependent; however, development after this stage absolutely requires follicle-stimulating hormone (FSH) and LH (Richards, 1975). Some recruited follicles are destined to undergo atresia while others, "dominant" follicles, survive an environment suppressive to the growth of other follicles and eventually ovulate (Goodman and Hodgen, 1983). This pattern of growth appears to be continuous, resulting in an ever-present pool of follicles available for ovulation when needed (Lang, 1965).

Both FSH and LH, the primary gonadotropins involved in folliculogenesis (Hisaw, 1947), are produced by the anterior pituitary. After binding to specific plasma membrane receptors in preantral follicles, gonadotropins induce cyclic adenosine-3',5'-monophosphate (cAMP)-mediated enhancement of steroidogenic enzyme activity, and thus steroid accumulation (especially that of estradiol) in follicular fluid and release of this hormone to the general circulation. Estradiol is the key steroid involved in promoting folliculogenesis (Pencharz, 1940; Richards,1975) and synthetic estrogens have been shown to greatly enhance the effects of FSH in promoting folliculogenesis in rats (Goldenberg *et al.*, 1972).

Receptors in the Dominant Follicle

Much of the physiology of folliculogenesis is determined by changes in the ability of theca and granulosa cells to respond to various hormonal and nonhormonal factors. Hence a discussion of the evolutionary changes in receptor concentrations in granulosa and theca cells as the follicle matures is appropriate. Granulosa cells of preantral follicles possess FSH receptors, but none for LH while the reverse situation exists for theca cells. Follicle stimulating

hormone acting on preantral follicles induces its own receptors as well as receptors for LH in granulosa cells at the time of antrum formation (Zeleznik *et al.*, 1974; Rajaniemi *et al.*, 1977). Receptors for LH are found on thecal cells from early stages of follicle development (Hillier, 1987). Human chorionic gonadotropin (hCG), whose biological properties resemble that of LH, progressively increases LH receptors in granulosa and theca in small antral follicles of the rat (Richards and Bogovich, 1982); however, theca cells never acquire FSH receptors (Carson *et al.*, 1979; Richards, 1980). Ability of FSH to induce its own and LH receptors in granulosa cells of preantral follicles appears to require the presence of theca cells and their simultaneous stimulation by LH (Lindner *et al.*, 1977; Hillier, 1987). Furthermore these data are supported by the observation that the analog of the FSH-induced second messenger cAMP, 8-Br-cAMP, did not induce receptors for these peptide hormones in granulosa cells cultured in the absence of theca cells (Lindner *et al.*, 1977). Apparently, theca cells produce a substance(s) that acts in concert with FSH to induce gonadotropin receptors in granulosa cells.

The classic marker for a preovulatory (Graafian) follicle is the LH receptor. In healthy ovine (Carson *et al.*, 1979) or porcine (Nakano *et al.*, 1977) follicles, hCG binding to LH receptors is related directly to follicle size. On the other hand, FSH binding decreases with follicle growth. The observed increases in LH receptor concentration as follicles increase in size are largely due to changes in the population of receptors in granulosa, rather than in theca cells (Carson *et al.*, 1979). Preovulatory sheep follicles with only theca LH receptors were found to have significantly higher antral fluid testosterone levels than those follicles with LH receptors in both the theca and the granulosa cells, which had higher estradiol levels (England *et al.*, 1981b). The latter follicles

were responsible for the preovulatory increase in estradiol secretion and were assumed to be the ovulatory follicles (England *et al.*, 1981b). Channing and Kammerman (1973) found that granulosa cells from large porcine follicles (greater than 5 mm diameter) bound 10 to 1000-fold more hCG than cells from small follicles (1-2 mm diameter). Moreover, in this species LH receptor numbers have been reported to increase from 300 in small follicles to 10,000 in large, preovulatory follicles, while the number of theca LH receptors only doubled (Hafez *et al.*, 1980). These changes in cell populations of LH receptors are consistent with the role of this hormone in causing ovulation.

Second Messenger Systems Involved in Folliculogenesis

Mechanisms by which various hormones exert their effects in the follicle are often the same mechanisms that exist in the corpus luteum. Hormones that affect the follicle appear to do so via the production of cAMP or the metabolites arising from the hydrolysis of phosphatidyl inositol. Both modes of hormone action have been found to be increasingly important in follicle and corpus luteum development and function during the past decade.

Gonadotropin-receptor binding activates the adenylate cyclase-cAMP system in plasma membranes (Richards and Bogovich, 1982), and hence causes protein kinase-mediated phosphorylation of various cytoplasmic enzymes and proteins. This process is mediated by the regulatory subunit of adenylate cyclase (the G_S protein) which exists in the unactivated state as a heterotrimer of an α, β and γ subunit. The β and γ subunits form the regulatory dimer of the G_S protein, and the α subunit, to which GDP is bound, has intrinsic GTPase activity. The hormone-receptor complex interacts with and activates the G_S protein by facilitating the replacement of GDP with GTP and the dissociation

of the regulatory dimer. This pathway depends on the presence of magnesium. By mechanisms yet to be defined, the α -GTP subunit stimulates the catalytic subunit of adenylate cyclase causing conversion of ATP to cAMP, which then activates various cellular kinases. The α subunit then hydrolyzes the bound GTP to GDP and reassociates with the $\beta\gamma$ subunit, rendering the G_S protein inactive (Birnbaumer *et al.*, 1985; Dunlap *et al.*, 1987). The adenylate cyclase system of cultured preovulatory rat follicles can become refractory to sustained high concentrations of LH and FSH (Zor *et al.*, 1976). It is possible that high episodic pulses of these gonadotropins during folliculogenesis and just prior to ovulation may lead to permanent desensitization of this second messenger system. This refractory state of the cAMP system may contribute to the demise of some follicles, yet other follicles may have means of protection from atresia (Ireland, 1987).

Gonadotropin releasing hormone (Ma and Leung, 1985; Davis *et al.*, 1986b) and LH (Davis *et al.*, 1984, 1986a) have been found to induce phosphoinositide metabolism in rat granulosa cells. Products of this pathway are involved in arachidonic acid liberation for prostaglandin synthesis, protein kinase C activation, calcium mobilization and guanylate cyclase activation (for reviews see Nishizuka, 1984; Nishizuka, 1986). Activation of protein kinase C in rat granulosa cells by phorbol ester increases prostaglandin E accumulation as well as increasing progesterone accumulation (Kawaii and Clark, 1985). Luteinizing hormone, but not FSH, dibutyryl cyclic AMP or a gonadotropin releasing hormone (GnRH) analog, activates phosphoinositide metabolism in porcine granulosa cells (Dimino and Snitzer, 1986). Furthermore, Veldhuis and Demers (1986) found that activation of protein kinase C in swine granulosa cells selectively inhibited cholesterol side chain cleavage activity without

adversely affecting cAMP generation or the aromatization of androgens to estrogen. These investigators later found that activation of the protein kinase C system in porcine granulosa cells also stimulated production of $PGF_{2\alpha}$ (Veldhuis *et al.*, 1987). It is possible that protein kinase C may modulate the actions of gonadotropic hormones in rats and hinder follicle development by adversely affecting steroidogenesis in swine. The stimulation of $PGF_{2\alpha}$ synthesis by this second messenger system may also be involved in ovulation. Nevertheless, the significance of this pathway in folliculogensis is not clear.

Steroidogenesis in the Developing Follicle

Because the two primary cell types of the follicle of domestic animals differ in receptor concentration and enzyme activities, interaction of LH-stimulated theca cells and FSH and LH-stimulated granulosa cells is required for full steroidogenic function. During folliculogenesis LH induces androgen synthesis via activation of theca cell side-chain cleavage enzymes, 17α -hydroxylase cytochrome P-450 (P-$450_{17\alpha}$) and 17-20 desmolase. Subtle increases in LH and cAMP stimulate synthesis of 17α -hydroxyprogesterone (Richards *et al.*, 1987). In general, granulosa cells are unable to produce androgens, but FSH stimulates increased aromatase activity in these cells and causes the conversion of thecal androgens to estrogens in the rat (Dorrington *et al.*, 1975; Fortune and Armstrong, 1978), sheep (Baird, 1977; Moor,1977) and pig (Evans *et al.*, 1981) . The pig is an exception because in this species both theca and granulosa cells are involved in estrogen production (Evans *et al.*, 1981). Thus in the pig, the role of FSH in regulating estrogen biosynthesis may be of lesser importance relative to its role in other species.

The induction of aromatase activity by FSH in granulosa cells, which occurs after thecal androgen synthesis (McNatty *et al.*, 1984), appears to be a key maturation step in folliculogenesis. In cattle, only a few of the 20-60 antral follicles producing androgens each day of the cycle are able to convert them to estradiol (McNatty *et al.*, 1984). Preovulatory LH peaks initially increase both estradiol and androgen secretion into the utero-ovarian vein in sheep (Baird, 1978; Baird *et al.*, 1981) followed by a 10-fold increase in estradiol with no further androgen secretion. Thus it appears that aromatase (Moon *et al.*, 1975; Baird *et al.*, 1981) and substrate availability may be the limiting factors in estradiol production (Baird *et al.*, 1981).

The androgen-estrogen ratio in follicular fluid has been used as an indicator of follicular physiologic integrity and viability in sheep: high concentrations of androgen are normal in small, healthy follicles, but is indicative of atresia in large follicles (Moor *et al.*, 1978).

In the granulosa cell, FSH and LH also cause lipoprotein uptake, cholesterol liberation, mobilization and conversion of cholesterol to pregnenolone via the mitochondrial side-chain cleavage enzymes. These gonadotropins also stimulate activation of 3ß-hydroxysteroid dehydrogenase, an enzyme that converts pregnenolone to progesterone. Injections of hCG into rats stimulated follicular progesterone production *in vitro* (Richards and Bogovich, 1982). The rate-limiting enzyme responsible for converting cholesterol to pregnenolone, cholesterol side-chain-cleavage cytochrome P-450 ($P\text{-}450_{scc}$), was detected in both bovine thecal and granulosa cells (Rodgers *et al.*, 1986a). Progesterone is secreted by both theca and granulosa cells, although granulosa cells of the ovine secrete more of this hormone than theca cells (Moor, 1977).

Selection and Dominance

Selection of an ovulatory follicle may begin to occur as early as the preantral phase of follicular development and continued growth of the follicle is dependent upon its ability to respond to available gonadotropins.

Dominant follicles may secrete factors that allow their own survival or cause the atresia of other follicles (Ireland *et al.*, 1979; Matton *et al.*, 1981). An example of such factors may be inhibin and estradiol and their relationship with FSH. Estradiol is the key steroid involved in promoting folliculogenesis (Pencharz, 1940; Richards,1975). Hence, the dominant follicle must have a greater capacity than other follicles for estradiol synthesis and secretion and must retain a functional estradiol receptor system in order to remain responsive to this steroid (Richards *et al.*, 1976). In cattle the dominant follicle has been found to contain a markedly greater inhibin bioactivity than other follicles (Padmanabhan *et al.*, 1984). The gonadotropin FSH induces production of both inhibin and estradiol, which in turn reduce FSH secretion from the pituitary (Padmanabhan *et al.*, 1984). Elevated estradiol concentrations in dominant follicles markedly enhance FSH and LH action (Hisaw, 1947; Richards, 1980). Thus estradiol may act locally within the follicle to allow for its survival while it acts systemically, along with inhibin, to provide an FSH-deficient environment in which other follicles cannot survive (Ireland, 1987).

Ovulation

Increasing estrogen synthesis by the ovulatory follicle eventually feeds back to the hypothalamus to initiate a release of an ovulatory surge of LH from the pituitary (Kesner *et al.*, 1981). Exposure of the follicle to LH results in a series of biochemical events that ultimately lead to physical rupture of the

ovulatory follicle and release of the ovum. The formation of prostaglandins, steroids and various proteins appears to be intimately associated with the process of ovulation.

The Role of Prostaglandins in Ovulation

Prostaglandins appear to be requisite for ovulation because prostaglandin synthase (prostaglandin endoperoxidase synthase; cyclooxygenase) inhibitors can prevent ovulation in rats (Orczyk and Behrman, 1972; Parr, 1974) and pigs (Ainsworth *et al.*, 1979) and synthesis of these 20 carbon arachidonic acid derivatives has been found to coincide with ovulation in rabbits (LeMaire *et al.*, 1973; Yang *et al.*; 1973) and rats (Bauminger and Linder, 1975). In rats, preovulatory but not small antral follicles produce large quantities of prostaglandins E (PGE) and $F_{2\alpha}$ when incubated with FSH and LH (Bauminger *et al.*, 1975; Richards and Bogavitch, 1982). Granulosa cells are the primary source of these prostaglandins (Erickson *et al.*, 1977; Clark *et al.*, 1978) and synthesis may result from prostaglandin synthase synthesis since a latency period of several hours and a slight increase in enzyme activity occurred with LH-induced stimulation of prostaglandins (Bauminger and Lindner, 1975; Clark *et al.*, 1978). This enzyme was not found in small antral or preovulatory rat follicles, but was elevated 40-fold in granulosa cells of luteinizing follicles 7 hours after an hCG injection (Richards *et al.*, 1987). Effects of LH in the rat were found to occur at a step in the prostaglandin synthesis pathway after arachidonic acid ester hydrolysis (Clark *et al.*, 1978) but did not depend upon steroid synthesis (Bauminger *et al.*, 1975). Although ovulation is obviously an inflammatory-proteolytic reaction (Parr, 1974), the exact role of prostaglandins is not yet known. Nevertheless, it has been found that $PGF_{2\alpha}$

stimulates synthesis, release and activation of a collagenase-like ovarian enzyme (Marsh and LeMaire, 1973; LeMaire and Marsh, 1975). It has also been observed that $PGF_{2\alpha}$ contributed to the rupture of lysosomes at the follicular apex and both $PGF_{2\alpha}$ (Espey, 1980) and PGE (Strickland and Beers, 1976) stimulated plasminogen activator production. Thus these prostaglandins are involved in stimulating proteases and reducing the strength of the follicle wall (for a review see Espey, 1980).

The Role of Steroid and Protein Synthesis during Ovulation

Steroid and protein synthesis also appear to be associated intimately with ovulation. Inhibition of steroidogenesis by several inhibitors before 6 hours after the LH surge in rats inhibited ovulation (Lipner and Greep, 1971). In addition actinomycin D, which prevents RNA synthesis, and two protein synthesis inhibitors puromycin and cycloheximide were able to prevent ovulation in rabbits *in vivo* (Pool and Lipner, 1966), suggesting a role for protein synthesis. The effects of cycloheximide were reversible by hCG injection. Furthermore, only puromycin caused visible cell damage suggesting that responses to all the other inhibitors were specific and not the result of generalized follicle damage.

The need for protein synthesis prior to ovulation may be explained by enzymatic degradation of the ovarian follicle wall. First, collagenolytic activity in rabbit and sow follicular tissue was observed to decrease prior to ovulation, while it increased in follicular fluid (Espey and Rondell, 1968). Shortly after, fibroblastic microvesicles extruding from rabbit theca externa and tunica albuginea plasma membranes in ovulatory follicles were found to secrete a substance that digests the surrounding extracellular matrix (Espey, 1971).

These early studies eventually led to the discovery of plasminogen, plasminogen activators, protease inhibitors and proteolytic activity due to plasmin found in bovine follicular fluid, and plasminogen activator in follicle wall homogenates (Beers, 1975). In addition these researchers showed that plasmin decreased bovine follicle wall tensile strength and, in a related study, that rat granulosa cells contained high levels of plasminogen activator that peaked prior to ovulation (Beers *et al.*, 1975). Furthermore they found that LH, dibutyryl cAMP (Beers *et al.*, 1975), $PGF_{2\alpha}$ (Espey, 1980) and PGE (Strickland and Beers, 1976) could stimulate plasminogen activator release. Plasminogen activator converts plasminogen to plasmin which in turn converts procollagenase to collagenase which degrades the follicle wall and assists in ovulation (Espey, 1980).

The Role of Ovarian Contractility in Ovulation

The role of ovarian contractility in ovulation has been debated and examined for some time. Several investigators noted the presence of myoid-like cells in follicles of various species, including ewes (O'Shea, 1971) and cows (Walles *et al.*, 1975a), but evidence for true muscle cells is both contradictory and eqivocal. Motility and cellular filaments were thought to be indicative of myoid tissue, but these characteristics have been observed in some thecal cells and fibroblasts (Espey, 1978). Prostaglandin $F_{2\alpha}$ and oxytocin have been found to stimulate ovarian contractility in rabbits (Virutamasen *et al.*, 1972) and ewes (O'Shea and Phillips, 1974). In contrast, prostaglandin E (PGE) was found to inhibit ovarian contractility in rabbits (Virutamasen *et al.*, 1972) and ewes (O'Shea and Phillips, 1974). The effects of steroids on contractility are unclear. Intrinsic contractility has also been

observed in ovine (O'Shea and Phillips, 1974) and bovine ovaries (Walles *et al.*, 1975b), but the time of the cycle in which it occurs conflicts with a role in ovulation. Other evidence for a role of contractility in the ovulatory process stems from the discovery of parasympathetic and sympathetic nerves in the ovary (Espey, 1978). However, this innervation is concentrated in medullary areas and terminates on blood vessels. Although alpha-adrenergic and cholinergic stimulation can intensify ongoing ovarian contractions in ewes (O'Shea and Phillips, 1974) and cows (Walles *et al.*, 1975b), and sympathomimetic agents produce dose-dependent contractions in bovine follicular wall strips (Walles *et al.*, 1975a), denervated rabbit ovaries retain the capacity to ovulate (Weiner *et al.*; 1975). Hence there is no conclusive evidence that ovarian contractions are requisite for ovulation, and the significance of this contractility is not yet defined.

Luteinization

Luteinization is the process by which a follicle gives rise to a corpus luteum. Events intrinsic to luteinization include those in which granulosa cells stop dividing (Rao *et al.*, 1978), enlarge, gain lipid droplets and shift steroidogenesis from predominantly estrogen production to synthesis of large quantities of progesterone. As opposed to granulosa cells, theca cells are stimulated to proliferate at this time (Rao *et al.*, 1978). Luteinization will occur only in preovulatory follicles that are exposed to an LH surge with a resultant increase in cAMP levels (Richards *et al.*, 1987). However, unlike ovulation, luteinization in the rat (Richards, 1975) and gilt (Ainsworth *et al.*, 1979) does not appear to depend on prostaglandin synthesis. Addition of LH or FSH to swine granulosa cells from medium-sized follicles in culture can promote their

luteinization as evidenced by increased epithelioid growth, lipid droplet and eosinic granule accumulation in the cytoplasm and increased progestin secretion. The effects of both LH and FSH are synergistic and prolactin (PRL) has no effect on this event (Channing, 1970).

Receptor Changes during Luteinization

The ovulatory LH surge has widespread effects on various receptor systems within the follicle. In the immature intact rat LH-induced luteinization following FSH priming was associated with increased labeled hCG uptake *in vivo* as well as increased granulosa cell LH receptor numbers (Rajaniemi *et al.*, 1977). On the other hand administration of ovulatory doses of LH to hypophysectomized rats primed with FSH that have large preovulatory follicles produced a rapid and marked attenuation of gonadotropin (Richards *et al.*, 1976) and estradiol receptor concentrations (Richards, 1975). This response is similar to that observed in ovulatory follicles of the ewe; both thecal and granulosa cells exhibited fewer LH receptors after the peak of the preovulatory gonadotropin surge (Webb and England, 1982). This decrease in gonadotropin receptors is likely due to internalization of the hormone-receptor complex (Conn *et al.*, 1978). Synthesis of receptors for low density lipoproteins, which contain progesterone precursors, and mRNA for these lipoprotein receptors, although detectable in ovine antral follicles, was greater in corpora lutea than these follicles (Rodgers *et al.*, 1987b). Thus it seems that although the young corpus luteum has fewer LH receptors, it appears to be better equipped to receive steroid precursors. Therefore the corpus luteum may be better able to respond to stimulation by LH with increased progesterone production.

Changes in Second Messenger Systems during Luteinization

It seems puzzling that one second messenger, cAMP, can have effects that vary with its concentrations and with stage of follicular development. Relatively low levels of cAMP produced in response to LH and FSH during folliculogenesis are associated with follicle growth and differentiation, but the high levels of cAMP produced in the Graafian follicle in response to the preovulatory LH surge trigger ovulation and luteinization. The answer to this dichotomy of function may lie in the types of regulatory units for cAMP-dependent protein kinase present at the time of the signal (for a review, see Richards *et al.*, 1987). For example, one type of regulatory subunit for this kinase, RII_{51}, is induced *in vivo* in granulosa cells by the synergistic actions of FSH and estradiol. However, RII_{51} and its mRNA are dramatically decreased in response to the preovulatory LH surge. Meanwhile, concentrations of another type of kinase regulatory subunit, RI, and its corresponding mRNA remain constant throughout follicular development and luteinization. After the preovulatory LH surge, however, RI is more predominant than RII_{51}. It is possible that elucidation of the mechanisms by which regulatory subunits of protein kinase A are affected by varying levels of cAMP or by sequential stimulation by gonadotropins and estradiol may lead to an explanation of how one second messsenger can have different effects in the follicle and possibly the corpus luteum.

Changes in Steroidogenesis during Luteinization

As previously mentioned steroidogenesis is drastically altered during luteinization. This process includes an overall shift of the major goal of the tissue from estrogen production to progesterone synthesis. Hours after an ovulatory LH surge, *in vivo* and(or) *in vitro*, estradiol concentrations in ovine follicular fluid (England *et al.*, 1981a) and estradiol secretion decline (Bjersing *et al.*, 1972; Moor, 1974; Baird *et al.*, 1981; Staigmiller *et al.*, 1982; Webb and England, 1982). Dieleman *et al.* (1983) found that bovine follicular fluid estradiol concentrations, nearly 16-fold and 73-fold higher than those of progesterone and testosterone, respectively, prior to the LH surge, decreased rapidly within 6 hours of the surge and were less than one tenth of their original levels by 20 hours (approximately four hours before ovulation). In the latter study, testosterone concentrations in bovine follicular fluid increased for the first 2 hours after the LH surge and then declined to very low levels; progesterone doubled in the next 6 hours, decreased to original levels by 10 hours and began to increase again by 20 hours (Dieleman *et al.*, 1983). Initial attenuation of steroidogenesis may be the result of an inability of the follicle to synthesize cAMP. In rabbits an ovulatory dose of hCG caused a decline in LH-induced cAMP accumulation within 30 minutes that continued until ovulation (Marsh *et al.*, 1973).

Alterations in steroidogenesis during luteinization also appear to be reflections of changes in various steroidogenic enzymes and mRNA for these enzymes. Because FSH, testosterone and androstenedione levels after the LH surge initially remain high *in vivo* (Baird *et al.*, 1981) and exogenous testosterone *in vitro* has no effect on estradiol secretion (Webb and England, 1982), the attenuation of estrogen production is likely due to decreased

aromatase activity, which may arise from the lack of LH receptors. While aromatase is the first enzyme to be affected by the LH surge, the 17, 20-side-chain-cleavage (desmolase) capacity is also inhibited within hours of the LH surge in ewes (Seamark *et al.*, 1974). It appears that subtle increases in LH and cAMP (as observed during folliculogenesis) stimulate synthesis of 17α - hydroxyprogesterone from progesterone, the rate-limiting step in androgen synthesis, yet the ovulatory LH surge and resulting elevated cAMP levels prevent synthesis of this steroid (Richards *et al.*, 1987). This theory is supported by the observation that, in large follicles of the cow, P-450$_{17\alpha}$ activity is high (Rodgers *et al.*, 1986a,c), but in the corpus luteum this activity is undetectable (Rodgers *et al.*, 1986c). Increased progesterone synthesis found in luteinizing follicles also appears to be the result of the synthesis of the inner mitochondrial membrane enzyme P-450$_{scc}$ (Richards *et al.*, 1987). Both P-450$_{scc}$ (Rodgers *et al.*, 1986b,c) and its electron donor adrenodoxin increased dramatically by the early-midluteal phase and remain active in small and large cells of the bovine corpus luteum until luteolysis (Rodgers *et al.*, 1986c). Ubiquitous non-steroidogenic inner mitochondrial enzymes, cytochrome c oxidase and F_1-ATPase, increased only several-fold, reflecting an increase in numbers of mitochondria (Rodgers *et al.*, 1986c). Thus the induced synthesis of steroidogenic enzymes during luteal formation was specific, and did not merely reflect mitochondrial biogenesis.

Changes in the above steroidogenic enzymes consistently parallel mRNA levels for these enzymes. For example, mRNA for P-450$_{17\alpha}$ were high in bovine antral follicles, low in young corpora lutea and undetecable in mature corpora lutea, as was the case with their transcript enzymes (Rodgers *et al.*, 1987b). Conversely, levels of mRNA P-450$_{scc}$ (Goldring *et al.*, 1987; Rodgers

et al., 1987b) and its electron donor adrenodoxin, were higher in bovine corpora lutea than in follicles (Rodgers *et al.*, 1987b). The positive correlations between these latter enzymes and their mRNA were found throughout the life of the corpus luteum, except during luteal regression when none of the previously mentioned mRNA were detectable (Rodgers *et al.*, 1987b).

Activity of enzymes responsible for synthesis of progesterone precursors are also affected by luteinization. The rate limiting enzyme in cholesterol synthesis, 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA), which is at low but detectable levels in medium and large bovine follicles has been reported to increase 50-fold in corpora lutea by the midluteal phase of the cycle (Rodgers *et al.*, 1987a). This enzyme then declines with the onset of luteolysis (Rodgers *et al.*, 1987a). Synthesis of this enzyme in bovine luteal cell culture is stimulated by dibutyryl cAMP and inhibited by bovine high density lipoprotein (Rodgers *et al.*, 1987a).

Luteinization in the rat also appears to be associated with an increase in granulosa, theca and interstitial cells in the activity of glucose-6-phosphate dehydrogenase (G6PD) and 20α -hydroxysteroid dehydrogenase (20α -OHD) in ovarian tissues (Kidwell *et al.*, 1966). Glucose-6PD, localized only in theca and interstitial cells of nonluteinizing preovulatory rat ovaries, catalyzes the first in a series of reactions resulting in glucose-6-phosphate oxidation. The enzyme 20α -OHD, which is undetectable in preovulatory rat ovaries, results in the production of 20α -hydroxypregn-4-en-3-one, a metabolite of progesterone. Furthermore, in the ewe 3-ß-hydroxysteroid dehydrogenase (3ß-OHD), present in thecal cells only at ovulation, was detected in granulosa cells 24-48 hours after LH was added to the incubation media (Moor *et al.*, 1975). Pupkin *et al.* (1966) found that although 3ß-OHD and G6PD were present in the rat Graafian

follicle, 20α -OHD was found only in the corpus luteum. In addition, diphosphopyridine nucleotide-dependent lactic dehydrogenase and 3ß-OHD increased in corpora lutea during their formation and declined thereafter, while triphosphopyridine nucleotide-dependent G6PD and 20α -OHD increased continuously throughout formation and involution of the corpus luteum (Pupkin *et al.*, 1966).

Oxytocin Synthesis in the Luteinizing Follicle

A final event associated with luteinization appears to be an increase in the synthesis of the peptide oxytocin (OT). Some investigators have found OT in the granulosa cells of small and large ovarian follicles (Kruip *et al.*, 1985), as well as in follicular fluid (Schams *et al.*, 1985). Although other investigators did not detect these low levels of OT, it is apparent that OT concentration (Kruip *et al.*, 1985; Wathes *et al.*, 1986) and release (Jungclas and Luck, 1986) from luteinizing granulosa cells and follicles (Schams *et al.*, 1985) is markedly enhanced. Luteinizing bovine granulosa cells *in vitro* also produce a polypeptide, bovine neurophysin 1, known to be part of the OT precursor molecule (Geenan *et al.*, 1985). Furthermore, cells from large follicles were observed to synthesize more OT and neurophysin than those from small follicles (Geenan *et al.*, 1985). Release of OT was also stimulated by liver, kidney, muscle and adrenal cortex and medulla-conditioned media; thus the factor capable of stimulating the release of OT has a widespread occurrence (Jungclas and Luck, 1986). Oxytocin concentration in ovine granulosa cells has been shown to increase for the 2 to 3 days after the ovulatory LH surge (Wathes *et al.*, 1986).

The Corpus Luteum

Large and Small Luteal Cells

The composition of the corpus luteum of a number of mammalian species has been found to consist of two populations of morphologically and endocrinologically distinct cells. These have been referred to as "small" and "large" luteal cells. In the young bovine corpus luteum (days 4-6), large luteal cells are predominantly derived from follicular granulosa cells and small luteal cells are mostly of thecal origin (Alila and Hansel, 1984). As the bovine corpus luteum ages, the percentage of large luteal cells increases as a result of theca-derived cell growth (Donaldson and Hansel, 1965) and conversion (Alila and Hansel, 1984). Bovine granulosa-derived cells undergo no further divisions after day 4 of the estrous cycle (Donaldson and Hansel, 1965) and disappear during early pregnancy (Alila and Hansel, 1984). It is not known with certainty whether the latter observations are the result of cell degeneration or the loss of the surface antigens used to detect cell origin (Alila and Hansel, 1984). Thecal-derived luteal cells persist throughout pregnancy (Alila and Hansel, 1984). In the ovine, there is an overall increase in the size of steroidogenic cells during the estrous cycle (Schwall *et al.*, 1986) but only small luteal cells increase in number (Farin *et al.*, 1986).

Effects of Luteinizing Hormone in the Corpus Luteum

Luteinizing hormone has been generally accepted as the major luteotropic hormone in domestic species. In the bovine, LH has been shown to stimulate progesterone synthesis in incubated luteal slices (Mason *et al.*, 1962) and to prolong the estrous cycle by extending the life of the corpus luteum

(Donaldson and Hansel, 1965). Similarly, Donaldson *et al.* (1965) found that administration of LH could overcome the inhibitory effects of exogenous OT in the cow. Luteinizing hormone has been shown to prolong the life span of the corpus luteum in intact (Kaltenbach *et al.*, 1968b; Karsch *et al.*, 1971) and hypophysectomized ewes (Kaltenbach *et al.*, 1968a). The effects of LH in the porcine are less dramatic than in the previous two species. In the gilt, hCG had no effect on progesterone synthesis by luteal tissue *in vitro* (Duncan *et al.*, 1961). Nevertheless, this gonadotropin induced a rapid secretion of progesterone as well as prolonged *de novo* synthesis of the steroid in superfused pig luteal tissue (Watson and Wrigglesworth, 1975). In addition, mares treated with antiserum against equine LH and FSH had smaller corpora lutea than control mares (Pineda *et al.*, 1973). Finally, LH also appears to be the primary luteotropin in the woman (Mais *et al.*, 1986) and the monkey (Hutchison and Zeleznik, 1984), yet the role of this hormone is diminished in laboratory species such as the rat, mouse and hamster. In these latter species, PRL serves as the major luteotropin (for a review, see Stormshak *et al.*, 1987).

Luteinizing Hormone Receptors in the Corpus Luteum

Numbers of luteal LH receptors in the ewe (Diekman *et al.*, 1978), sow (Ziecik *et al.*, 1980) and mare (Roser and Evans *et al.*, 1983) are highly correlated with progesterone secretion during the estrous cycle.

In the past decade it has been suggested that luteal LH receptors exist primarily on small luteal cells. Evidence for this assertion lies in two groups of observations. Firstly, under basal conditions, large luteal cells of the bovine (Urseley and Leymarie, 1979), superovulated (Fitz *et al.*, 1982) and naturally cycling ovine (Rodgers and O'Shea, 1982; Harrison *et al.*, 1987) and

porcine (Lemon and Loir, 1977) produce more progesterone than small luteal cells. Secondly, small luteal cells, however, appear to be more responsive to stimulation by LH in the cow (Donaldson and Hansel, 1965; Urseley and Leymarie, 1979), superovulated (Fitz *et al.*, 1982) and naturally cycling ewe (Rodgers and O'Shea, 1982; Hoyer *et al.*, 1984) and sow (Lemon and Loir, 1977). It has recently been shown that equal numbers of LH receptors are present in large and small cells during days 10 and 15 of the estrous cycle of the ewe (Harrison *et al.*, 1987). Nevertheless ovine large luteal cells do not secrete increased amounts of progesterone in response to LH (Harrison *et al.*, 1987). Small luteal cells collected from superovulated ewes also appear to be more responsive to cAMP agonists such as dibutyryl cAMP (Fitz *et al.*, 1982), cholera toxin and forskolin (Hoyer *et al.*, 1984). However, a lack of LH receptors, whether actual or an experimental artifact, does not explain completely the inability of luteotropins to stimulate progesterone production by large luteal cells. Although ovine LH was ineffective in raising intracellular levels of cAMP in large luteal cells from superovulated ewes, substances that dramatically increased concentrations of this second messenger (cholera toxin and forskolin) did not alter progesterone secretion (Hoyer *et al.*, 1984). In contrast to the situation in the ovine, bovine large luteal cells can respond to high levels of LH with increased progesterone synthesis (Urseley and Leymarie, 1979; Alila *et al.*, 1988). However, it should be noted that this response is limited and a fraction of the response of small luteal cells. In addition, bovine large luteal cells can also respond to a limited extent to forskolin and 8-Br-cAMP (Alila *et al.*, 1988). Because bovine small luteal cells develop into large luteal cells and still retain their LH receptors (Alila and Hansel, 1984), it is not known whether the former reponse to this gonadotropin

is due to species variation or if it is the result of cell conversion. Nevertheless, it appears that at least some bovine large luteal cells contain LH receptors and the appropriate functional second messenger systems.

Second Messengers Involved in Actions of LH on the Corpus Luteum

As in the ovarian follicle LH exerts its effects in the corpus luteum via adenylate cyclase and increased cAMP production (Marsh *et al.*, 1966; Pierce and Parsons, 1981). However, a loss of responsiveness of adenylate cyclase to LH is associated with the onset of regression (Anderson *et al.*, 1974; see subsequent discussion on The Role of Prostaglandin $F_{2\alpha}$ in Luteolysis).

In the past few years it has been discovered that LH (Davis *et al.*, 1987a) and hCG (Davis *et al.*, 1986c) evoke phosphatidyl inositol hydrolysis and increase intracellular levels of calcium in isolated bovine luteal cells. These results indicate that the phospholipase C pathway (see subsequent discussion on Second Messengers Involved in the Action of $PGF_{2\alpha}$: the Phospholipase C Pathway) may be involved in some of the actions of LH. The observations that LH stimulates not one but two different second messengers in the same cells is indeed curious. It is also puzzling that both luteolytic hormones such as $PGF_{2\alpha}$ and luteotropic hormones such as LH produce different effects in the same tissue and yet use common second messengers. The significance of the phospholipase C pathway with respect to the effects of LH on the corpus luteum is likely to be the focus of much future research.

Luteotropic Prostaglandins

Receptors for prostaglandins are found predominantly on large luteal cells of the superovulated ewe (Fitz *et al.*, 1982). Prostaglandin E_2

administration in ewes has been shown to delay natural (Pratt *et al.*, 1977), $PGF_{2\alpha}$ -induced (Henderson *et al.*, 1977) and estradiol-induced luteal regression. Although this prostaglandin prolonged the estrous cycle, it had no direct stimulation of *in vivo* progesterone secretion in the ewe (Pratt *et al.*, 1977) or the cow (Chenault, 1983). Nevertheless, prostaglandins E_1, E_2, and I_2 later were found to stimulate progesterone secretion in large but not small ovine luteal cells obtained from superovulated animals (Fitz *et al.*, 1984a). Despite these previous observations, Rodgers *et al.* (1985) and Harrison *et al.* (1987) did not observe any effect of PGE_2 in large or small ovine luteal cells obtained from naturally cycling animals.

Second Messengers Involved in Actions of Luteotropic Prostaglandins on the Corpus Luteum

Ability of luteotropic prostaglandins to stimulate progesterone production in large luteal cells was independent of cAMP production and enhanced adenylate cyclase activity (Fitz *et al.*, 1984a). Nevertheless, prostaglandins E_1 and E_2 have been found to stimulate increases in inositol phosphate hydrolysis and intracellular calcium levels in dispersed bovine luteal cells (Davis *et al.*, 1987b). Thus it appears that the phospholipase C/protein kinase C pathway may be involved in the effects of these prostaglandins in the corpus luteum (see subsequent discussion on Second messengers involved in the action of $PGF_{2\alpha}$: the phospholipase C pathway).

The Role of Prostaglandin $F_{2\alpha}$ in Luteolysis

Prostaglandin $F_{2\alpha}$ of uterine origin is generally accepted as the major physiological luteolysin in domestic animals. Although the effects of

administering this prostaglandin are sometimes confusing and the exact mechanisms of its action are not fully understood, luteolytic effects of $PGF_{2\alpha}$ are well documented. Hysterectomy prolongs the life span of the corpus luteum in the cow, ewe (Wiltbank and Casida, 1956) gilt (Moeljono *et al.*, 1976) and guinea pig (Loeb, 1927). A similar effect is observed in ewes and cows that have been immunized against PGF (Fairclough *et al.*, 1981). Furthermore, $PGF_{2\alpha}$ injections were luteolytic in intact (Schramm *et al.*, 1983) and hysterectomized ewes (Gengenbach *et al.*, 1977) and in pregnant (Diehl and Day, 1974), estradiol benzoate-treated (Kraeling *et al.*, 1975) and hysterectomized gilts (Moeljono *et al.*, 1976).

Prostaglandin $F_{2\alpha}$ decreases plasma and luteal progesterone during the midluteal phase of the estrous cycle of cows (Weston and Hixon, 1980) and ewes (Diekman *et al.*,1978; Fitz *et al.*, 1980; Agudo *et al.*, 1984). Decreases in bovine serum progesterone after prostaglandin administration were followed by decreases in luteal progesterone concentrations, and weight of corpora lutea in the ewe (Diekman *et al.*, 1978; Agudo *et al.*, 1984) and cow (Weston and Hixon, 1980). Similar to its effects *in vivo*, $PGF_{2\alpha}$ has also been shown to inhibit LH-stimulated progesterone secretion by luteal tissue of the cow, ewe and gilt *in vitro* (for a review see Behrman, 1979).

Effects of $PGF_{2\alpha}$ on Luteal Gonadotropin Receptors

Final effects of $PGF_{2\alpha}$ include changes in luteal receptors for LH. Although exogenous $PGF_{2\alpha}$ did not impair binding of LH to the ovine luteal receptor (Diekman *et al.*, 1978), it caused a marked reduction in numbers of total and occupied receptors in the cow (Spicer *et al.*, 1981) and the ewe (Diekman *et al.*, 1978; Agudo *et al.*, 1984).

Second Messengers Affected by $PGF_{2\alpha}$ in the Corpus Luteum

Administration of $PGF_{2\alpha}$ to ewes attenuates luteal basal and stimulated cAMP levels by decreasing basal adenylate cyclase activity (Agudo *et al.*, 1984). This alteration in cAMP occurs prior to decreases in serum and luteal progesterone. It appears that $PGF_{2\alpha}$ accomplishes this by increasing the activity of the cAMP-degrading enzyme phosphodiesterase and also by preventing the activation of adenylate cyclase by GTP, LH and GTP plus LH (Agudo *et al.*, 1984). Thus it seems that prevention of interactions between the regulatory and catalytic subunits of adenylate cyclase is critical in the alteration of progesterone synthesis and secretion by $PGF_{2\alpha}$.

Investigators who examined the effects of $PGF_{2\alpha}$ *in vitro* have strengthened the previous supposition regarding the actions of $PGF_{2\alpha}$ and have provided further insight into the mechanisms by which this compound exerts luteolytic effects. Prostaglandin $F_{2\alpha}$ has been found to reduce the final equilibrium level of bound LH in rat luteal cells by blocking LH-induced up-regulation of cryptic LH receptors (Luborsky *et al.*, 1984a) and to reduce LH receptor aggregation (Luborsky *et al.*, 1984b). Dorflinger *et al.* (1983) found that inhibition of progesterone release by $PGF_{2\alpha}$ *in vitro* occurred in rat luteal cells incubated with dibutyryl cAMP, the adenylate cyclase activator cholera toxin, or cells preactivated by LH. In contrast to the observations of Agudo *et al.* (1984) who administered $PGF_{2\alpha}$ to ewes, Fletcher and Niswender (1982) reported that $PGF_{2\alpha}$ *in vitro* had no effects on basal progesterone accumulation or adenylate cyclase activity in ovine luteal tissue. These latter researchers did find, however, that $PGF_{2\alpha}$ *in vitro* decreased LH-stimulated progesterone secretion and cAMP levels without altering phosphodiesterase activity. These investigators also demonstrated that $PGF_{2\alpha}$ had little effect on

the ability of forskolin and cholera toxin to induce cAMP accumulation. Thus it appeared that $PGF_{2\alpha}$, in addition to preventing interaction between the occupied LH receptor and the nucleotide-binding component on adenylate cyclase, caused a post-receptor, cAMP-independent lesion of progesterone secretion (Fletcher and Niswender, 1982).

Inconsistencies in the Effects of $PGF_{2\alpha}$ on the Corpus Luteum

Reported effects of $PGF_{2\alpha}$ on luteal function are inconsistent and often confusing, a possible result of variations in the stage of the estrous cycle studied and the time of observations relative to $PGF_{2\alpha}$ administration. For example, $PGF_{2\alpha}$ administered during the midluteal phase of the estrous cycle caused a transient increase in progesterone secretion prior to a decrease in venous levels of this hormone in cows (Schallenberger *et al.*, 1984) and increased levels of luteal progesterone in ewes (Agudo *et al.*, 1984). Low doses of $PGF_{2\alpha}$ injected into the ovarian bursae of pseudopregnant hamsters markedly increased ovarian progesterone, while high doses were luteolytic (Lukaszewska et al., 1972). In addition, $PGF_{2\alpha}$ frequently has been found to have no effect on *in vitro* basal progesterone concentrations in rat luteal cells (Behrman, 1971; Dorflinger et al., 1983) and ovine luteal slices (Fletcher and Niswender, 1982). It has also been found to increase progesterone production by the bovine corpus luteum (Speroff and Ramwell, 1970; Sellner and Wickersham, 1970; Hansel et al., 1973; Weston and Hixon, 1980).

The observation that exogenous $PGF_{2\alpha}$ is effective in inducing luteolysis only in the latter two-thirds of the estrous cycle (Hansel and Convey, 1983) is also puzzling. It is known that the cells that comprise the ovarian follicle can respond to this compound--indeed $PGF_{2\alpha}$ is requisite for ovulation

(for a review, see Espey, 1980). Furthermore, the second messenger system commonly observed to be activated by $PGF_{2\alpha}$ has been found to be functional in granulosa cells of the rat (Davis *et al.*, 1984; Kawai and Clark, 1985; Davis *et al.*, 1986a,b) and gilt (Dimino and Snitzer, 1986; Veldhuis and Demers, 1986). The answer to this question may lie in the relationship that develops in the mature corpus luteum between $PGF_{2\alpha}$ and oxytocin in which each regulates the secretion of the other (see subsequent discussion on A Role for Oxytocin in Luteolysis).

Effects of $PGF_{2\alpha}$ on Small and Large Luteal Cells

The seemingly contradictory effects of $PGF_{2\alpha}$ may be explained in part by the ability of this hormone to affect different types of cells within the ovary. For example, $PGF_{2\alpha}$ was found to increase progesterone synthesis in small (theca-derived) but not large (granulosa-derived) bovine luteal cells, while it attenuated LH-, 8-Br-cAMP- and forskolin-stimulated synthesis of this steroid in large cells (Alila *et al.*, 1988). Prostaglandin $F_{2\alpha}$ has an inhibitory effect on progesterone production in large luteal cells in both superovulated (Fitz *et al.*, 1984b) and normally cycling ewes (Rodgers *et al.*, 1985), but not in small luteal cells (Rodgers *et al.*, 1985).

It has been postulated that the luteolytic effect of $PGF_{2\alpha}$ requires interactions between the two cell populations. Oxytocin does not appear to be the crucial mediator in this process, however, because it does not alter progesterone production by either small or large luteal cells (Rodgers *et al.*, 1985). Although ovine large luteal cells after $PGF_{2\alpha}$ injection become more fragile, luteolysis in the ewe is associated with a preferential loss in the number of small luteal cells (Farin *et al.*, 1986; Schwall *et al.*, 1986; Bradin *et al.*, 1988).

These changes in cell populations occur several hours after the decline in serum progesterone levels.

Receptors for $PGF_{2\alpha}$ in the Corpus Luteum

Receptors for prostaglandins $F_{2\alpha}$ and E are predominantly found on large luteal cells of the superovulated ewe (Fitz *et al.*, 1982). It is not known whether this is the case in the normal cycling ewe, but the fact that this prostaglandin can stimulate inositol phosphate production in both large and small bovine luteal cells (Davis *et al.*, 1988) indicates the presence of $PGF_{2\alpha}$ receptors in both cell populations of the cow.

Second Messengers Involved in the Action of $PGF_{2\alpha}$: the Phospholipase C Pathway

The mechanism by which $PGF_{2\alpha}$ acts on the corpus luteum may involve activation of protein kinase C. A variety of hormones have been found to activate protein kinase C through stimulation of phosphoinositide hydrolysis, mainly effected by activation of the enzyme phospholipase C. These hormones bind to plasma membrane receptors, which then cause a G protein-mediated activation of phospholipase C. This enzyme acts on phosphatidylinositol in the plasma membrane to yield inositol triphosphate and diacylglycerol. Inositol triphosphate acts within the cell to release calcium stores and thus increase intracellular calcium levels. Diacylglycerol, in the presence of at least normal intracellular calcium levels activates protein kinase C, which in turn phosphorylates other proteins requisite for evoking cellular responses to the hormone (for reviews, see Nishizuka *et al.*, 1984; Downes and Mitchell, 1985; Majerus *et al.*, 1986; Gilman, 1987).

Researchers have demonstrated that, in dispersed rat luteal cells (Raymond *et al.*, 1983; Leung *et al.*, 1986), bovine large and small luteal cells (Davis *et al.*, 1987b, 1988), and ovine luteal slices (McCann and Flint, 1987), $PGF_{2\alpha}$ stimulated inositol phosphate accumulation, a result of phosphoinositide hydrolysis and thus an indication of protein kinase C activation. This compound had no effect on cAMP levels in small or large bovine luteal cells during 30 minutes of incubation (Davis *et al.*, 1988). Moreover, a phorbol ester known to directly activate this kinase was found to have identical effects as $PGF_{2\alpha}$ in small bovine luteal cells (Alila *et al.*, 1988).

Support for the assertion that $PGF_{2\alpha}$ causes its effects in the corpus luteum via the phosphatidylinositol cascade, was provided by Davis *et al.* (1987b) who found that this prostaglandin mobilized intracellular calcium in addition to stimulating increased inositol phospholipid hydrolysis. Furthermore, many researchers have noted that high intracellular calcium levels cause the same effects as $PGF_{2\alpha}$. For example, Dorflinger *et al.* (1984) found that calcium ionophore A23187 caused a marked, dose-related and extracellular calcium-dependent decrease in LH-stimulated cAMP levels and progesterone secretion in rat luteal cells, without affecting the ability of LH to bind to its receptor. In this latter study, A23187 did not affect basal cAMP and progesterone secretion unless present at extremely high concentrations in the incubation media. In the former studies calcium might be acting to decrease the binding of GTP to the G-protein and(or) the release of GDP-binding protein from adenylate cyclase (Behrman *et al.*, 1985). Moreover, the observations that extracellular-calcium concentrations do not alter the effects of $PGF_{2\alpha}$ within rat luteal tissue point toward a role of intracellular calcium (Behrman *et al.*, 1985).

Oxytocin in the Corpus Luteum

An indication that OT of luteal origin may play an important role in regulating uterine and(or) ovarian function during the estrous cycle was precipitated by observations that plasma levels of this peptide varied in a cyclical manner that roughly paralleled plasma progesterone levels and overall luteal function. Highest OT concentrations in the bovine peripheral plasma occurred during the early and midluteal phases of the estrous cycle (Schams, 1983). Systemic concentrations of OT in the ovine begin to increase on days 3 and 4 (Schams and Lahlou-Kassi, 1984) and remain high during the rest of the early or midluteal phase of the estrous cycle (Sheldrick and Flint, 1981; Webb *et al.*, 1981; Mitchell *et al.*, 1982; Schams *et al.*, 1982). These peptide levels decreased on days 13 to 15 of early pregnancy in the ewe, about 2 days earlier than in nonpregnant animals (Sheldrick and Flint, 1981; Webb *et al.*, 1981; Schams and Lahlou-Kassi, 1984). After days 18 to 19 of ovine pregnancy OT levels remain at low, follicular phase levels until term (Schams and Lahlou-Kassi, 1984). Further evidence that the corpus luteum may contribute to systemic OT levels was provided by the observation that levels of this hormone were correlated to the number of corpora lutea present in ewes (Schams *et al.*, 1982; Schams *et al.*, 1985).

Subsequently ovine (Wathes and Swann, 1982; Watkins *et al.*, 1986) bovine (Fields *et al.*, 1983) and caprine (Homeida, 1986) corpora lutea were found to contain large quantities of OT identical to that produced by the hypothalamus. Oxytocin was detected immunocytochemically in large, but not small luteal cells of the cow (Guldenaar *et al.*,1984) and ewe (Rodgers *et al.*, 1983; Sawyer *et al.*, 1986) where it exists in a population of secretory granules

(Rice and Thorburn, 1985; Theodosis *et al.*, 1986; Fields and Fields, 1986). Luteal concentrations of bovine OT, which parallel systemic levels, are highest on days 8 to 12 of the cycle and decline before luteal progesterone levels late in the estrous cycle (Wathes *et al.*, 1984; Kruip *et al.*, 1985; Schams *et al.*, 1985; Abdelgadir *et al.*, 1987; Fehr *et al.*, 1987). Levels of luteal OT are significantly lower in pregnant cows (Wathes *et al.*, 1983; Schams *et al.*, 1985) and ewes (Sheldrick and Flint, 1983b).

Luteal Oxytocin Synthesis

Detection of large amounts of neurophysin, a protein cosynthesized with OT and thought to serve as its carrier, as well as OT in the bovine corpus luteum led to the hypothesis that the ovary was not simply sequestering OT, but rather was synthesizing this hormone (Wathes *et al.*, 1983). Further evidence for luteal OT synthesis was provided when Flint and Sheldrick (1982a,b) found significant veno-arterial OT concentration differences across ovaries containing corpora lutea but not in those without corpora lutea. Definitive proof that ovine and bovine corpora lutea do indeed produce oxytocin and that this biosynthesis is similar to that in the hypothalamus was provided by Swann *et al.* (1984) who incubated luteal cells in the presence of [^{35}S]cysteine and isolated labeled OT and a labeled precursor with a similar electrophoretic mobility to that of rat-hypothalamic OT precursor.

Oxytocin mRNA appears to be virtually identical in the hypothalamus and the ovary (Ivell and Richter,1984); with the exception that luteal mRNA contains a shorter polyadenosine tail (Ivell and Richter,1984). During the bovine estrous cycle the corpus luteum produces approximately 250 times more OT mRNA than a single hypothalamus (Ivell and Richter,1984). Like the

peptide, mRNA encoding luteal OT is confined to the large luteal cells of the bovine where it reaches maximal levels during the first 6 days of the cycle and generally peaks on day 3 (Ivell *et al.* 1985; Fehr *et al.*,1987). In contrast hypothalamic OT mRNA concentrations do not vary in cycling cows (Ivell *et al.*,1985). By day 7 of the cycle luteal OT mRNA levels decline rapidly, presumably a reflection of the short half-life of most eucaryotic mRNA's (Fehr *et al.*,1987). Luteal OT mRNA, transcribed from a single gene comprised of 3 exons, is translated to a precursor polypeptide of approximately 12,800 Da (Ivell *et al.*,1985). Immediately adjacent to the N-terminal signal sequence of this polypeptide is OT followed by neurophysin I (Ivell *et al.*,1985). Young bovine luteal cells must lack the requisite enzymes for post-translational processing because it is not until the cells are fully mature that they can complete OT synthesis and packaging, which generally occurs by day 7 of the cycle (Fehr *et al.*,1987).

Alteration of Oxytocin Synthesis

Administration of progesterone inhibits and estrogen potentiates OT release during vaginal distension in the ewe (Roberts and Share, 1969). Luteinizing hormone *in vitro* has no effect on OT secretion in incubated slices of ovine (Hirst *et al.*, 1986) and bovine (Abdelgadir *et al.*, 1987) corpora lutea. Similarly, prostaglandin E_2 had no effect on OT secretion by bovine luteal tissue (Abdelgadir *et al.*, 1987). However, release from ovine luteal tissue was stimulated by the presence of potassium and it was also dependent on calcium (Hirst *et al.*, 1986). Incubation in calcium-free medium attenuated OT secretion, while calcium ionophore enhanced secretion of this hormone (Hirst *et al.*, 1986). Incubation with PGE_1, hCG, human PRL, or dibutyryl cAMP did not affect

volume occupancy of OT-containing granules in bovine large luteal cells (Chegini and Rao, 1987).

A Role For Oxytocin in Luteolysis

In recent years it has been speculated that OT and $PGF_{2\alpha}$ work together to enhance the secretion of each other and induce luteolysis. This was supported by observations that, OT could induce the release of uterine $PGF_{2\alpha}$, and this prostaglandin could induce the release of the ovarian peptide.

Effects of Exogenous Oxytocin during the Estrous Cycle

It has long been known that exogenous OT can alter the duration of estrous cycle. Daily administration of natural or synthetic OT to cycling heifers during the first week of the estrous cycle shortened the cycle from the usual 21 days to approximately 8 days (Armstrong and Hansel, 1959; Hansel and Wagner, 1960; Anderson *et al.,* 1965). However, OT had no effect on plasma progesterone until day 5, after which steroid levels were attenuated (Donaldson *et al.,* 1970). Cycles following the OT-treated cycles, however, were normal in duration (Armstrong and Hansel, 1959). Although exogenous OT can alter the cycle of the intact bovine, it produces less dramatic results in the ovine. Milne (1963) treated ewes with OT at a much higher dose per body weight than that used in the cow (Armstrong and Hansel, 1959) during the first 6 days of the cycle. Although morphological changes in luteal cells similar to those observed in heifers were noted, this treatment failed to hasten luteolysis in the ewes (Milne, 1963).

Effects of Immunization Against Oxytocin

Further evidence for a role of OT in luteolysis was provided by the finding that active and passive immunization of ewes against OT prolonged the luteal phase of the estrous cycle as evaluated by estrous behavior and quantitation of progesterone levels (Sheldrick *et al.*, 1980; Schams *et al.*, 1983). Although the response was variable, animals that responded to treatment had low circulating concentrations of free OT (Schams *et al.*, 1983).

Effects of Oxytocin on $PGF_{2\alpha}$ Release

Luteolytic effects of OT appear to be mediated by the ability of this peptide to stimulate uterine release of $PGF_{2\alpha}$. Preliminary evidence for involvement of the uterus as a mediator of OT action on luteal regression was the observation that OT was luteolytic in partially hysterectomized heifers (Anderson *et al.*, 1965) and in those that had undergone sham hysterectomy, but was ineffective in totally hysterectomized heifers (Armstrong and Hansel, 1959; Anderson *et al.*, 1965). Further evidence for such action of OT was provided by Ginther *et al.* (1967) who found that OT was luteolytic in unilaterally hysterectomized heifers in which the corpus luteum was adjacent to the retained uterine horn, but not when it was on the opposite side. Moreover, subcutaneous OT has been shown to increase bovine uterine vein $PGF_{2\alpha}$ levels within 30 to 240 minutes (Milvae and Hansel, 1980). However, these investigators failed to demonstrate that OT-induced $PGF_{2\alpha}$ release from the uterus was correlated with levels of $PGF_{2\alpha}$ in the ovarian vein and thus they found no evidence for a transfer of prostaglandin from the uterine vein to the ovarian artery (Milvae and Hansel, 1980). However, Oyedipe *et al.* (1984) found that subcutaneous OT injections on days 2 through 6 increased plasma

levels of the major $PGF_{2\alpha}$ metabolite 15-keto-13,14-dihydro $PGF_{2\alpha}$ (PGFM) and speculated that perhaps the inability of Milvae and Hansel (1980) to detect $PGF_{2\alpha}$ in the ovarian vein may have been due to its short half-life in blood. Despite the less-than-dramatic effects of OT on the ovine estrous cycle, this peptide can nevertheless elevate utero-ovarian venous levels of PGF in pregnant ewes (Mitchell *et al.*, 1975). Subcutaneous administration of OT to dairy goats on days 3 to 6 of the estrous cycle induced luteolysis and a significant increase in peripheral plasma concentrations of PGFM within 30 minutes of the injections (Cooke and Homeida, 1982). Oral administration of the prostaglandin synthase inhibitor meclofenamic acid prevented the luteolytic action of OT and the OT-induced increase of $PGF_{2\alpha}$ metabolites (Cooke and Homeida, 1983). Active immunization against OT, which was able to prolong the estrous cycle in some goats from a normal average of 19 days to 29 days, induced very low levels of systemic PGFM with no marked pulsatile secretion similar to that observed in control goats (Cooke and Homeida, 1985). In addition intra-arterial administration of an OT-antagonist from days 12 to 20 delayed luteal regression from day 20 to 26 and thus interaction between OT and its uterine receptors appears requisite for $PGF_{2\alpha}$ release and luteolysis in the goat (Homeida and Khalafalla, 1987).

Effects of $PGF_{2\alpha}$ on Oxytocin Release

Early evidence leading to the hypothesis that $PGF_{2\alpha}$ regulates OT release from the corpus luteum was provided by Sheldrick and Flint (1983a). These researchers first noted that luteal OT concentrations were dramatically attenuated in hysterectomized ewes, suggesting that OT synthesis and, or secretion might be influenced by some uterine factor (Sheldrick and Flint,

1983a). Moreover, cloprostenol, an analog of $PGF_{2\alpha}$, when administered intramuscularly to ewes on days 11 to 14 of the estrous cycle decreased luteal OT levels and rapidly increased ovarian venous OT, with maximal concentrations occurring by 20 minutes after injection (Flint and Sheldrick, 1982a,b). Vena cava and jugular vein concentrations of OT in the cow increased after a luteolytic injection of chloprostenol, and reached maximal levels by 5-10 minutes, after which time they declined steadily for 24-36 hours to below initial levels (Schallenberger *et al.*,1984). Chloprostenol had no effect on ovine pituitary secretion of OT (Flint and Sheldrick, 1982a,b), on plasma OT concentration in ovariectomized ewes (Flint and Sheldrick, 1982b) or on OT secretion in hysterectomized ewes (Sheldrick and Flint, 1983a). Immunoreactive neurophysin and OT concentrations after a luteolytic dose of $PGF_{2\alpha}$ in the cow increased to a greater extent in the vena cava than in the jugular vein or aorta, indicating that the source of these peptides was indeed the ovary and not the pituitary (Schams *et al.*, 1985). Additional evidence that $PGF_{2\alpha}$ regulates OT release from the corpus luteum was found when indomethacin, a prostaglandin synthase inhibitor, caused a marked reduction in the amplitude of oxytocin-associated neurophysin surges in the systemic plasma of ewes (Watkins *et al.*, 1984).

Chegini and Rao (1987) found that $PGF_{2\alpha}$ caused a rapid degranulation of electron-dense perinuclear granules of bovine large luteal cells of pregnancy that was significant within 10 minutes. These granules virtually disappeared from the cells after two hours of incubation in the presence of this prostaglandin (Chegini and Rao, 1987). Although some researchers found that $PGF_{2\alpha}$ did not alter OT secretion significantly during one hour of incubation, there was a nonsignificant increase in OT levels in the medium and

the detection of effects of the prostaglandin may not have been possible within the sensitivity limits of the assay methods used (Hirst *et al.*, 1986). Other researchers who utilized a more sensitive radioimmunoassay have found a direct stimulatory effect of this prostaglandin on OT synthesis and secretion from luteal tissue (Abdelgadir *et al.*, 1987).

Uterine Oxytocin Receptors

Oxytocin levels in systemic blood are low when $PGF_{2\alpha}$ is initially released and it is unlikely that increased ovarian secretion of OT is the cause for the release of this prostaglandin (Sheldrick and Flint, 1981; Webb *et al.*, 1981). However, increased effectiveness of the OT at the cellular level due to increased concentrations of uterine OT receptors may still be the causal factor in the initiation of luteolysis (Sheldrick and Flint, 1981). These latter researchers strengthened their hypothesis that OT receptor concentrations play a role in luteolysis with observations that mean OT receptor concentrations in the caruncular and intercaruncular endometrium and the myometrium of the ewe increased dramatically between days 10 to 13 of the cycle and the day of estrus (Sheldrick and Flint, 1985a). This increase of a single population of relatively unspecific uterine OT receptors coincided with luteolysis and declining serum progesterone, but occurred after the decline in systemic OT and after the rise of ovarian venous estradiol-17ß. Variations among receptor concentrations of the different tissues reflected their ability to synthesize $PGF_{2\alpha}$ (Sheldrick and Flint, 1985a). Thus levels of OT receptors were highest early in the cycle (when OT administration is most effective in inducing luteolysis), although at that time, only 1% would normally be occupied (Sheldrick and Flint, 1985a). Nevertheless, it is possible that OT, like other peptide hormones such

as LH and EGF may exert their effects at low receptor occupancy (Sheldrick and Flint, 1985a). Potency of OT to elicit a release of $PGF_{2\alpha}$ from the endometrium of the ewe, but not the myometrium *in vitro* increased as estrus approached and it was at this time that endometrial receptors for OT were at their highest levels (Roberts *et al.*, 1976).

Because increased numbers of uterine OT receptors coincide with estrus, it was postulated that estrogen might regulate the availability of endometrial OT receptors in domestic animals. Indeed, estradiol increased the numbers of myometrial oxytocin receptors while progesterone reversed the induction of OT receptors in ovariectomized, estradiol-treated and pregnant rabbits (Jacobson *et al.*, 1987). Estradiol-17ß also has been found to sensitize the uterus to the ability of OT to release $PGF_{2\alpha}$, although it does not affect circulating OT concentrations (Roberts *et al.*, 1975). Furthermore, while OT alone had negligible effects on $PGF_{2\alpha}$ levels in vena cava blood of anestrous ewes, OT administration to estradiol-primed ewes caused a dramatic increase in $PGF_{2\alpha}$ release (Sharma and Fitzpatrick, 1974). Finally, Sheldrick and Flint (1985b) found that intramuscular estradiol administration increased uterine OT receptor concentrations.

In later experiments, Flint and Sheldrick (1985) found that continuous intravenous infusions of OT into ewes (3µg/h) from days 13 to 21 prolonged the cycle by 7 days and prevented the rise in uterine OT receptors that normally precedes estrus. This treatment was not effective if initiated on day 14 after luteolysis had commenced or if chloprostenol was administered on day 15. Levels of PGFM were greatly attenuated during and following OT treatment (Sheldrick and Flint, 1987). The cycle of the heifer can also be extended with infusions of OT. Intravenous OT infusions from days 10 to 22 extended the

cycles from an average of 20.5 to 25.8 days (Gilbert *et al.*,1987). Thus continuous OT treatment may inhibit uterine $PGF_{2\alpha}$ production, possibly by down-regulating uterine OT receptors (Flint and Sheldrick, 1985). This theory is supported by observations that uterine OT receptors on day 16 of the cycle in nonpregnant ewes are over seven times higher than those of the pregnant ewe at that time and that nonpregnant animals are able to respond to OT by secreting over 12 times more $PGF_{2\alpha}$ into the uterine vein than pregnant ewes (McCracken *et al.*,1984). However, uterine refractoriness to OT-stimulated $PGF_{2\alpha}$ release was found not to be the result of down-regulated OT receptors (Sheldrick and Flint, 1985b).

McCracken's Theory for the Mechanism of Luteolysis

The previous observations concerning the ability of OT and $PGF_{2\alpha}$ to induce the secretion of the other, as well as those concerning changes in uterine OT receptors led McCracken *et al.* (1984) to formulate the following scenario for the mechanism of luteolysis in sheep. First, declining progesterone concentrations and increasing estrogen concentrations during the late luteal phase of the estrous cycle cause an increase in synthesis of uterine OT receptors. Oxytocin of pituitary and(or) ovarian origin act upon these receptors to initiate a release of $PGF_{2\alpha}$. This prostaglandin reaches the ovary via a local countercurrent transfer from the uterine vein to the ovarian artery. Oxytocin is released from the ovary in response to $PGF_{2\alpha}$ and acts upon the uterus to reinforce or regulate secretion of the prostaglandin. This cycle, in which OT and $PGF_{2\alpha}$ repeatedly stimulate the release of the other, continues until luteolysis is complete.

Paracrine Effects of Oxytocin in the Corpus Luteum

Direct effects of OT on the corpus luteum have been documented although results are equivocal and the physiological significance of these results is not well defined. Low concentrations of OT were found to significantly enhance basal progesterone production of luteal cells from pregnant cows (Tan *et al.*, 1982). Higher doses had no significant effect on basal progesterone but inhibited hCG-stimulated production of this steroid (Tan *et al.*, 1982). On the other hand, high concentrations of OT have inhibited basal and hCG-stimulated progesterone production of dispersed luteal cells from nonpregnant cows and estradiol may potentiate these inhibitory effects (Tan and Biggs, 1984). Oxytocin *in vitro* was found to have no effect on progesterone production in small or large ovine luteal cells (Rodgers *et al.*, 1985).

Evidence Against a Local Role for Oxytocin in the Luteolytic Process

Despite all previous observations suggesting otherwise, OT may not be requisite for natural luteolysis. Evidence that OT was not a crucial element of the luteolytic process was the finding that chloprostenol administration to hysterectomized ewes was as effective in inducing luteolysis as in intact ewes; hence luteal OT did not appear to be involved in intraluteal events mediating prostaglandin-induced luteolysis (Sheldrick and Flint, 1983a).

EXPERIMENTS 1 AND 2: BOVINE LUTEAL OXYTOCIN SYNTHESIS AND SECRETION *IN VITRO*: EFFECTS OF PHORBOL ESTER, CALCIUM IONOPHORE AND INDOMETHACIN

INTRODUCTION

Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) induces OT secretion from the bovine corpus luteum *in vivo* (Walters *et al.*, 1983; Schallenberger *et al.*, 1984) and *in vitro* (Abdelgadir *et al.*, 1987; Chegini and Rao, 1987). The mechanism by which $PGF_{2\alpha}$ promotes the release of luteal OT is not known. *In vitro* treatment of rat (Leung *et al.*, 1986), bovine (Davis *et al.*, 1987b) and ovine (McCann and Flint, 1987) luteal tissue with $PGF_{2\alpha}$ has been shown to provoke inositol phosphate accumulation, a result of phosphoinositide hydrolysis. In general, hormonally-stimulated phosphoinositide hydrolysis results in intracellular calcium mobilization and diacylglycerol production (Nishizuka *et al.*, 1984; Downes and Michell, 1985; Majerus *et al.*, 1986). Both calcium and diacylglycerol interact with protein kinase C to effect its activation (Nishizuka *et al.*, 1984). Thus $PGF_{2\alpha}$-induced secretion of OT may be due to changes in intracellular calcium levels and(or) protein kinase C activation. If this premise is true then agents that alter levels of intracellular calcium or activity of protein kinase C might alter luteal OT secretion.

Phorbol esters such as 12 -*0* -tetradecanoylphorbol-13-acetate (TPA) mimic the actions of diacylglycerol and can directly activate protein kinase C (Castagna *et al.*, 1982). Luteal cells of the rat (Kawai and Clark, 1985) and bovine (Brunswig *et al*., 1986) have been shown to respond to TPA with increased progesterone secretion. It is conceivable that TPA might also stimulate OT secretion by activating protein kinase C.

If changes in cellular calcium are intimately associated with OT synthesis or release, agents such as calcium ionophore A23187 and indomethacin might also provoke changes in the secretion of this peptide. Ovine luteal cells have been shown to respond to A23187 with increased OT secretion (Hirst *et al.*, 1986). The ionophore increases intracellular levels of calcium and has been shown to activate protein kinase C (Kaibuchi *et al.*, 1983). Indomethacin inhibits prostaglandin synthase (Vane, 1971; Smith and Lands, 1971; Flower, 1974), and alters intracellular calcium metabolism (Northover, 1985). Although indomethacin does not inhibit plasma membrane calcium transport (Burch *et al.*, 1983), it reduces levels of this ion in a variety of cells types (Northover, 1982; Burch *et al.*, 1983; Northover, 1985) rendering the ion unavailable as an intracellular messenger.

The present study was conducted to examine the effects of TPA, calcium ionophore A23187 and indomethacin on OT synthesis and release from the bovine corpus luteum *in vitro.* In addition, the effects of TPA on progesterone secretion by this tissue were examined.

MATERIALS AND METHODS

Animals and Corpora Lutea Collection

Six 18-month-old crossbred beef heifers were used for Exp. 1; five similar heifers were used in Exp. 2. Heifers were observed twice daily for estrus using vasectomized bulls. After exhibiting at least two consecutive estrous cycles of normal duration (18-23 days), heifers were restrained on day 8 of the cycle (day of estrus = day 0), tranquilized by injection of 3 ml acepromazine into the middle coccygeal vein and injected with 3 ml 2% lidocaine to induce caudal epidural anesthesia. The corpus luteum then was collected by colpotomy and immediately placed in 50 ml Ham's F-12 medium (4 C) prepared as described by Abdelgadir *et al.* (1987). It was transported to the laboratory (5 min) where it was freed of connective tissue, weighed and sliced (0.3 mm thickness). Tissue slices were washed four times with 50 ml of Ham's F-12, blotted on a filter paper and divided into aliquots.

Hormones and Chemicals

Indomethacin and TPA were purchased from Sigma Chemical Co. (St. Louis, MO) and [^{125}I]OT was purchased from New England Nuclear (Boston, MA). Calcium ionophore A23187 was purchased from Calbiochem (San Diego, CA). Rabbit anti-oxytocin serum was a generous donation of Dr. Dieter Schams of the Institute of Physiology, Technical University of Munich, Freising-Weihenstephan, West Germany.

Experiment One

Experiment 1 was conducted to examine the *in vitro* effects of two levels of TPA on OT synthesis and release, and one level of TPA on progesterone secretion from corpora lutea on day 8 of the estrous cycle. In addition, the effects of indomethacin alone and in conjunction with TPA on OT synthesis and release were examined. Tissue aliquots (255 $\pm$ 6 mg) were placed into siliconized incubation flasks (prepared in duplicate) containing 1.99 ml of Ham's F-12 incubation medium to which were added the following treatments: 1) vehicle, which consisted of 10 µl dimethylsulfoxide (DMSO; unincubated control); 2) vehicle (incubated control); 3) 0.81 nM TPA; 4) 1.62 nM TPA; 5) 100 µM indomethacin; 6) 1.62 nM TPA and 100 µM indomethacin. Concentrations of TPA and DMSO employed were less than those found to be toxic in porcine granulosa cells (Veldhuis and Demers, 1986) and bovine luteal cells (Brunswig *et al.*, 1986), respectively. These levels of TPA were found to activate protein kinase C directly *in vitro* in rat brain cells (Castagna *et al.*,1982). Although other investigators have used levels of TPA up to 200 nM, relatively low levels were utilized in the present study in an attempt to observe specific, nontoxic effects of this phorbol ester. The level of indomethacin utilized was among those found to inhibit calcium accumulation and prevent prostaglandin synthesis by hamster liver mitochondria and microsomes (Burch *et al.*, 1983). All flasks were flushed with 95% O_2 - 5% CO_2, stoppered and appropriate flasks were incubated in a Dubnoff incubator at 38 C for 2 h. Incubation was terminated by immersing flasks in an ice bath (4 C), after which the contents were transferred to plastic tubes and centrifuged at 3000 x g. The tissue pellet was separated immediately from the supernatant and both were

subsequently frozen and stored at -20 C pending extraction and quantification of OT and progesterone. Unincubated control flasks were processed similarly.

Experiment Two

Based upon the results of Exp. 1, in which responses to indomethacin were variable, a second experiment was conducted to re-examine the effects of indomethacin on OT release from corpora lutea *in vitro.* A second objective of this experiment was to examine the effects of the calcium ionophore A23187 on OT synthesis and release. Tissue aliquots (216.8 $\pm$ 4.9 mg) were placed into siliconized flasks (prepared in duplicate) containing 1.99 ml Ham's F-12 to which were added: 1) vehicle, 10µl DMSO (unincubated control); 2) vehicle (incubated control); 3) 100 µM indomethacin; 4) 0.3 µM A23187. This concentration of ionophore was shown to enhance protein kinase C activity by Kaibuchi *et al.* (1983). Tissue slices were incubated, centrifuged and frozen as in Exp. 1.

Extraction and radioimmunoassays

Oxytocin released into the incubation media was assayed directly without extraction. Tissue OT was extracted as described by Abdelgadir *et al.* (1987) and the mean extraction efficiency was 84.4 $\pm$ 1.7%. Oxytocin was measured by radioimmunoassay as described by Abdelgadir *et al.* (1987). Sensitivity of the assay was 0.25 pg/tube ($P<0.05$, $n = 32$). The intra- and interassay coefficients of variation were 8.0 (n=7) and 10.2% (n=9), respectively. Quantity of OT in media was considered to represent secretion and OT synthesis was estimated by subtracting initial tissue levels from the sum of the incubated tissue levels and the levels of OT secreted during incubation.

Progesterone extraction and radioimmunoassays were conducted as described by Koligian and Stormshak (1977). The mean extraction efficiency was 90.3 ± 4.0%. Sensitivity of this assay was 10 pg/tube ($P<0.05$, $n = 10$). Intra- and interassay coefficients of variation were 9.0 ($n=6$) and 4.3% ($n=5$), respectively.

Statistical analyses

Data involving effects of two concentrations of TPA on OT synthesis and secretion were analyzed statistically by analysis of variance for an experiment of randomized block design. Effects of indomethacin in combination with TPA were evaluated by use of split-plot analysis of variance for a experiment of 2x2 factorial design. Effects of TPA on progesterone secretion in Exp. 1, indomethacin in Exps. 1 and 2 and A23187 in Exp. 2 were analyzed by use of Student's paired t-test.

RESULTS

Experiment One

In vitro effects of TPA on luteal tissue levels of OT after incubation and on the quantity of OT released by luteal tissue are depicted in Fig. 1. Levels of OT released by unincubated tissue (46.3 ± 12.0 ng/g of tissue) were subtracted from the total quantity of OT released into incubation media. Initial levels of OT in sliced, washed luteal tissue were 390.3 ± 60.2 ng/g (Fig. 1). Although neither concentration of TPA altered tissue levels of OT during the 2 h incubation period, both levels of TPA caused a significant increase in OT secretion relative to the control ($P<0.01$, Fig. 1). While the higher level of TPA

tended to cause a greater secretion of OT, the effects of the two concentrations of phorbol ester on this characteristic did not differ. Synthesis of OT, as determined by subtracting initial tissue levels of OT from total OT after incubation (released OT + tissue OT), was significantly increased by both concentrations of TPA ($P<0.02$, Fig. 2). The higher level of TPA appeared to be more effective in increasing OT synthesis than the lower concentration; however, the difference in response was not significant.

Changes in luteal OT secretion in response to indomethacin may be seen in Fig. 3. While indomethacin did not cause a significant change in OT release, it inhibited the stimulatory effects of TPA (indomethacin x TPA interaction: $P<0.005$).

In addition to enhancing OT secretion, TPA (0.81 nM) caused an increase in progesterone release ($ng \cdot g^{-1} \cdot 2h^{-1}$; control, 1056.2; 0.81 nM TPA, 1333.3; common SE = 86.4; $P<0.025$). The TPA-induced secretion of progesterone and OT occurred concomitantly.

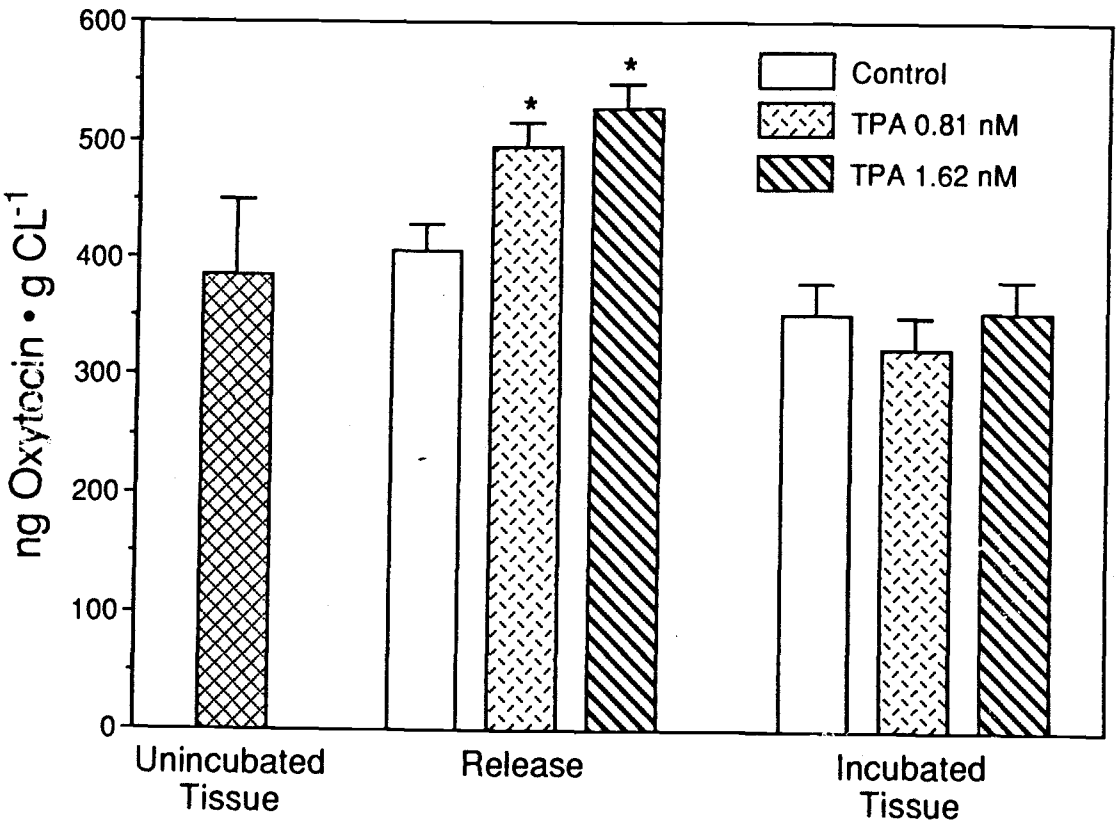

Fig. 1. Oxytocin concentrations in unincubated luteal tissue (mean ± SE) and in tissue and media (mean ± common SE) after 2 h incubation with TPA on day 8 of the estrous cycle.

*Different from control ($P<0.01$).

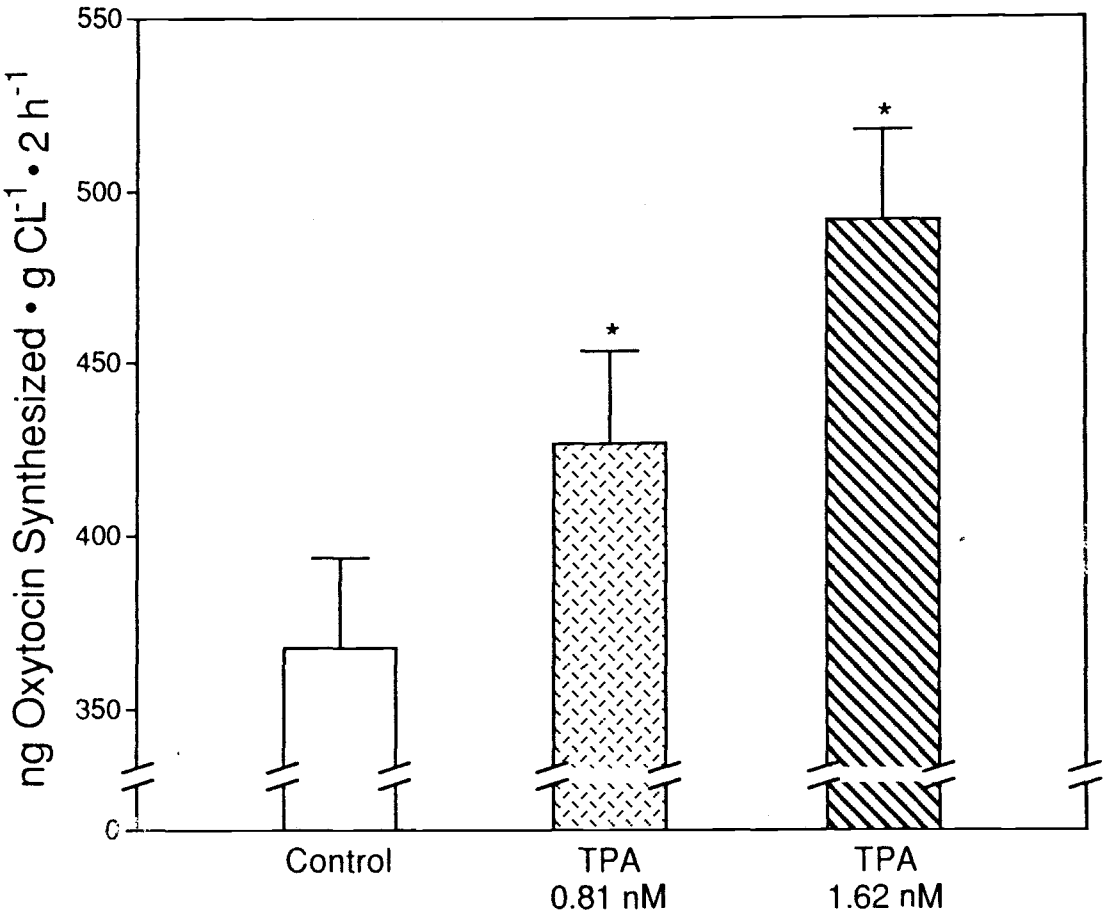

Fig. 2. Oxytocin synthesis (mean ± common SE; $P<0.02$) by luteal tissue during 2 h incubation with TPA on day 8 of the estrous cycle.
*Different from control ($P<0.02$).

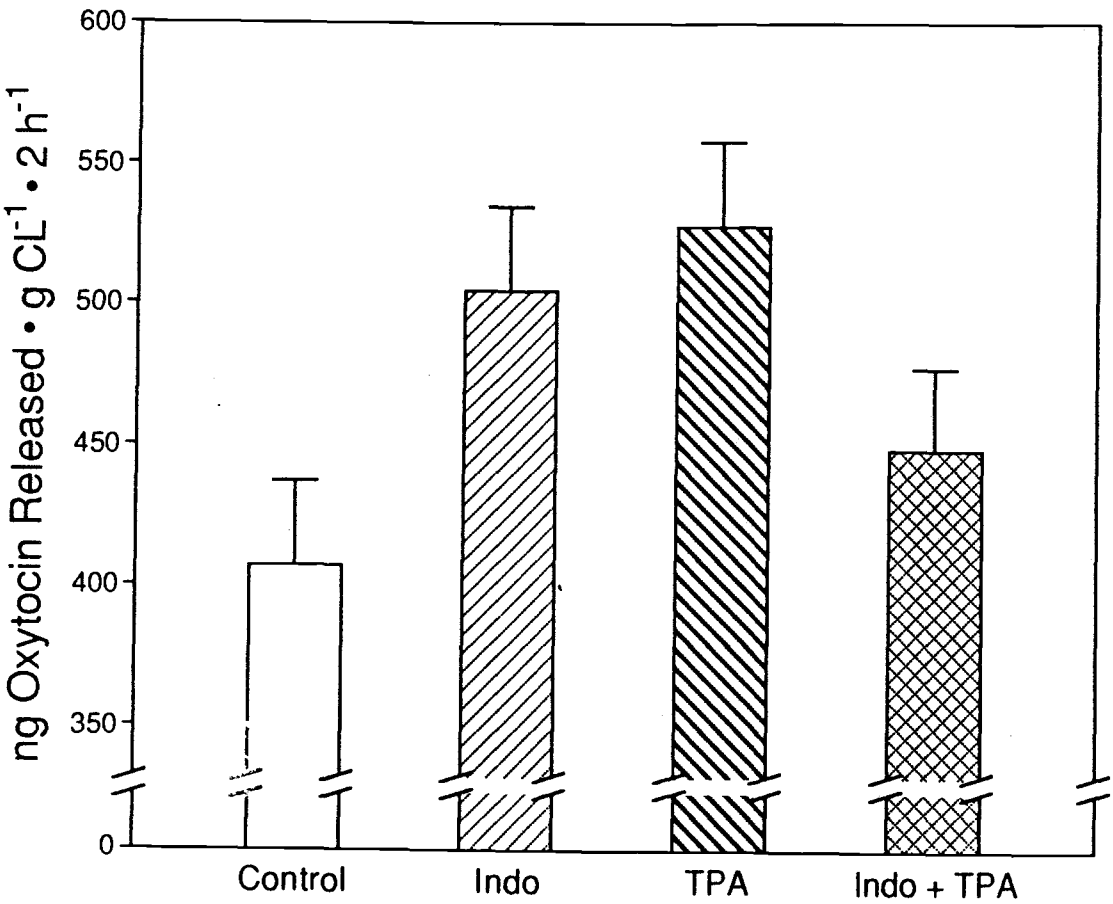

Fig. 3. Oxytocin release (mean ± common SE) by luteal tissue into media after 2 h incubation with 1.62 nM TPA and(or) 100 μM indomethacin (TPA x indomethacin interaction, $P<0.005$).

Experiment Two

Levels of OT released by unincubated tissue (18.7 ± 7.7 ng/g of tissue) were subtracted from the quantity of OT released during incubation. As in Exp. 1, indomethacin did not alter the release of OT (control: 248.9 $ng \cdot g^{-1} \cdot 2h^{-1}$; indomethacin: 290.4 $ng \cdot g^{-1} \cdot 2h^{-1}$; common SE = 19.5). Calcium ionophore A23187 significantly increased the secretion of OT ($P<0.01$; Table 1), and increased OT synthesis slightly ($P \approx 0.09$).

DISCUSSION

The acquired data indicate for the first time that the phorbol ester TPA stimulates OT synthesis and secretion from bovine luteal tissue *in vitro*. This phorbol ester is able to intercalate into the plasma membrane and, because of its structural similarity to diacylglycerol, directly activate protein kinase C (Castagna *et al.*, 1982; Nishizuka *et al.*, 1984). It is possible that TPA-activated protein kinase C played some role in the secretion of OT although effects of this phorbol ester via other intracellular pathways cannot yet be excluded. Although there is strong evidence that protein kinase C is the major receptor for TPA, it does not appear that this kinase exists as a single, homogeneous class of receptors (Blumberg, 1988). Nevertheless, support for a role of this kinase in luteal OT production was provided by the observation that phospholipase C, an enzyme that causes diacylglycerol production and thus protein kinase C activation, increased OT secretion from ovine luteal slices (Hirst *et al.*, 1988).

Table 1. Oxytocin released and synthesized by bovine luteal slices in response to incubation with calcium ionophore A23187

A23187 (μM)	n	Mean OT ($ng \cdot g^{-1} \cdot 2h^{-1}$)	
		Release[a]	Synthesis[a]
0 (control)	5	248.9	129.4
0.3	5	327.4[b]	165.6[c]

[a]Common estimate of the standard error = 16.0 (release); 16.4 (synthesis).
[b]$P<0.01$ compared to control.
[c]$P\approx0.09$.

Furthermore, this phospholipase-induced increase was not observed in the presence of $CoCl_2$, which inhibits calcium entry into cells (Hirst *et al.*, 1988). Because $PGF_{2\alpha}$ can stimulate phosphoinositide hydrolysis in bovine luteal cells (Davis *et al.*, 1987b), and if indeed protein kinase C is involved in OT synthesis and secretion, this enzyme might be a contributory factor in the stimulation of OT secretion *in vivo* and *in vitro* by $PGF_{2\alpha}$.

In the current experiments, OT concentrations did not differ among unincubated control, incubated control or TPA-treated tissues, indicating that OT release was accompanied by an increase in synthesis of this nonapeptide. Abdelgadir *et al.* (1988) found that during a 2 h incubation period OT secretion was due to a release of stored hormone. Thus OT synthesis observed in the present study apparently was not the result of increased OT mRNA translation, but rather post-translational processing of a prohormone. This possibility is also supported by the data of Ivell *et al.* (1985) who reported that OT mRNA concentrations were maximal on day 3 of the bovine oestrous cycle, had decreased by day 7 and were basal by day 11, which suggests that OT mRNA levels were low in day 8 corpora lutea used for the present study.

Results of the current study also indicate that the calcium ionophore A23187 can enhance OT secretion in the bovine corpus luteum. Similar effects of this compound on OT secretion by ovine luteal cells have been reported (Hirst *et al.*,1986). The actions of this ionophore are presumably a result of its ability to increase intracellular calcium levels. In other tissues A23187 has been shown to act synergistically with phorbol esters or diacylglycerol to enhance protein kinase C activation (Nishizuka *et al.*, 1984). It would be interesting to examine interactions between TPA and A23187 in order to

determine if the ionophore affects kinase C activity and thereby stimulates OT secretion from bovine and ovine luteal tissue.

Indomethacin, a compound shown to decrease intracellular calcium in nonsteroidogenic tissues (Northover, 1982), blocked the stimulatory effects of TPA on bovine luteal OT synthesis and release in the present study. It is possible that the suppression of TPA-induced OT secretion and synthesis by indomethacin reflects the ability of this compound to interfere with the availability and(or) utilization of calcium required for protein kinase C activation. The present data do not eliminate the possibility that indomethacin, which also inhibits cyclooxygenase (Vane, 1971; Smith and Lands, 1971), blocked TPA-induced synthesis of an intermediary cyclooxygenase product that in turn promoted OT release. However, the inhibitory effect of this compound on OT synthesis and secretion is not likely attributable to the blocked production of basal levels of prostaglandins because indomethacin alone failed to attenuate the release or synthesis of OT relative to control tissue. Indirect support for our assumption that prostaglandins were not involved is provided also by Burch *et al.* (1983) who found that the ability of indomethacin to inhibit calcium accumulation by rat and hamster liver mitochondria and microsomes was not dependent on inhibition of prostaglandins and could not be reversed by products of cyclooxygenase activity. The observation that aspirin, ibuprofen and indomethacin at doses inhibitory to cyclooxygenase had no effect on basal OT secretion by ovine luteal slices (Hirst *et al.*, 1988) lends credence to the previous assertion. In the latter study it is noteworthy that indomethacin, but not ibuprofen or aspirin, blocked arachidonic acid-stimulated OT secretion, indicating that the effects of indomethacin were not prostaglandin-dependent (Hirst *et al.*, 1988).

In our study TPA-induced OT and progesterone secretion by luteal tissue *in vitro* occurred concomitantly. These results are in agreement with those of Brunswig *et al.* (1986) who found that TPA levels comparable to those used in the present study increased progesterone release from dispersed bovine luteal cells. The observed increased progesterone production may be the result of effects exerted exclusively in large luteal cells or may require interactions between large and small luteal cells. Levels of TPA from one to 1000-fold those used in the present study were found to have no effect on basal progesterone release by dispersed bovine small luteal cells (Benhaim *et al.*, 1987). Effects of TPA on LH-stimulated progesterone production also appear to vary with cell type and both TPA and LH levels (Brunswig *et al.*, 1986; Benhaim *et al.*, 1987). Tumor-promoting phorbol esters also have been shown to be steroidogenic in other tissues. For example, Shemesh *et al.* (1985) found that TPA stimulated progesterone synthesis in bovine placental cells and calcium ionophore A23187 augmented this increase.

In conclusion it appears that the phorbol ester TPA and agents that specifically alter intracellular calcium levels lead to changes in luteal OT synthesis and release and progesterone secretion. The precise mechanism(s) by which each is able to evoke these responses needs to be delineated.

GENERAL DISCUSSION

Researchers investigating the corpus luteum have served to provide as many questions as answers regarding control and function of this organ. These questions include those involving regulation of corpora lutea on a systemic level as well as those concerning the molecular aspects of the mechanisms by which this organ functions.

One of the more intriguing aspects of corpus luteum function has been the discovery that it can synthesize and secrete OT. It is curious that quantities of mRNA for OT are apparently maximal several days prior to the time that tissue and serum levels of this hormone reach their zenith during the midluteal phase of the estrous cycle. Thereafter concentrations of OT steadily decline and are minimal at the time of onset of luteolysis. This sequence of events is perplexing, especially from the standpoint of defining a role for this hormone in the course of normal reproductive events.

Because tissue and serum levels of this peptide increase during the first one-half of the cycle, one would expect this peptide to exert some effect on ovarian and(or) uterine function at that time. It is possible that it does but the appropriate responses of these tissues have not yet been investigated. Levels of uterine OT receptors are lowest during the midluteal phase of the estrous cycle which might lead one to believe that this hormone is without effect on the uterus at that stage of the cycle. However, it is conceivable that OT, long known for its ability to evoke smooth muscle contractions, causes some changes in uterine motility required for transport of the blastocyst to an appropriate position within the uterus. This possibility could be investigated perhaps by use of an OT antagonist.

Increased synthesis of OT throughout the early part of the estrous cycle may be of importance in regulating differentiation and maturation of cells that eventually populate the mature corpus luteum. Although the ability of OT to alter progesterone synthesis remains equivocal, a paracrine role for OT may exist by which this peptide regulates cellular differentiation and the possible evolution of small luteal cells into large luteal cells.

The fact that $PGF_{2\alpha}$ promotes OT secretion from the corpus luteum has been amply demonstrated. The possibility that this prostaglandin evokes this secretion through activation of luteal phosphoinositide metabolism and protein kinase C is supported by the results of the present research. In the present study a tumor-promoting phorbol ester induced a release of OT. Because this phorbol ester has been shown to directly activate protein kinase C, and because $PGF_{2\alpha}$ has been found to cause similar responses in luteal cells of several species, it may be postulated that the mechanism by which this prostaglandin evokes OT synthesis and release involves this kinase. It would be interesting to examine the effects of other kinase C-activating phorbol esters, as well as the effects of inactive phorbol esters. It would also be intriguing to compare dose-related and maximal changes in the ability of both $PGF_{2\alpha}$ and tumor-promoting phorbol esters to induce OT synthesis and secretion, in addition to investigating interactions of these two compounds with respect to OT production. For example, a synergistic relationship between these two componds would be indicative of a separate modes of action. On the other hand, if TPA and $PGF_{2\alpha}$ increase OT secretion by a common pathway, cells in the presence of maximally-stimulating dose of one would not be expected to be subject to further stimulation by the other. Finally, it would be appropriate to

correlate the ability of TPA to activate protein kinase C and to induce release of OT from large luteal cells.

The fact that a calcium ionophore also provoked a release of OT serves as evidence that calcium mobilization may be involved in the secretion of this nonapeptide. It is interesting that indomethacin blocked the ability of phorbol ester to stimulate the secretion of OT. Although some evidence suggests that indomethacin can interfere with calcium mobilization, it is conceivable that this compound acts by some other mechanism to interfere with the process of exocytosis. Further research on the action of this compound in the luteal cell is needed.

In the past year it has been discovered that exogenous OT can induce both phosphoinositide hydrolysis and prostaglandin production in human amnion cells *in vitro* (Moore *et al.*, 1988). These results suggest that the mechanism by which OT evokes prostaglandin secretion from these cells involves the phospholipase C pathway. The possibility of that OT may act in a similar manner in other reproductive tissues (for example, the uterus) is quite intriguing and warrants further investigation. If this indeed proves to be the case, OT could indirectly control its own production and a circular pattern of release would exist. For example, OT could act on the uterus to induce phosphoinositide hydrolysis which could mediate the stimulation of $PGF_{2\alpha}$ secretion. Prostaglandin $F_{2\alpha}$ could then act upon the corpus luteum, stimulate phospholipase C, and therefore kinase C activation, and thereby induce OT secretion, which could feed back to the uterus. This circular pattern of feedback between the ovary and the uterus is the molecular basis for McCracken's theory of luteolysis. Unfortunately, this and McCracken's theories, however attractive, do not explain all aspects of luteolysis. The foremost discrepancy involves the

above mentioned observations that concentrations of OT and its mRNA decrease, not increase, during luteolysis.

Research that could prove useful in answering the above questions and discerning the mechanism of luteolysis and general luteal function would have to examine changes in small and large luteal cells as well as the corpus luteum as a whole. Too often in the past the corpus luteum has been examined as a static tissue, which it is not. Research on a molecular basis must emphasize varying stages of the estrous cycle. Perhaps then this complex and intriguing tissue will be more fully understood.

BIBLIOGRAPHY

Abdelgadir, S.E. 1988. Oxytocin Synthesis and Release by the Bovine Corpus Luteum. Ph.D. Dissertation, Oregon State University, Corvallis.

Abdelgadir, S.E., Swanson, L.V., Oldfield, J.E. and Stormshak, F. 1987. $PGF_{2\alpha}$ -induced release of oxytocin from bovine corpora lutea in vitro. Biol. Reprod. 37: 550-555.

Agudo, L.Sp., W.L. Zahler and M.F. Smith. 1984. Effect of prostaglandin $F_{2\alpha}$ on the adenylate cyclase and phosphodiesterase activity of ovine corpora lutea. J. Anim. Sci. 58:955-962.

Ainsworth, L., B.K. Tsang, B.R. Downey, R.D. Baker, G.T. Marcus and D.T. Armstrong. 1979. Effects of indomethacin on ovulation and luteal function in gilts. Biol. Reprod. 21:401-411.

Alila, H.W. and W. Hansel. 1984. Origin of different cell types in the bovine corpus luteum as characterized by specific monoclonal antibodies. Biol. Reprod. 31:1015-1025.

Alila, H.W., J.P. Dowd, R.A. Corradino, W.V. Harris and W. Hansel. 1988. Control of progesterone production in small and large luteal cells separated by flow cytometry. J. Reprod. Fertil. 82:645-655.

Anderson, L.L., A.M. Bowerman and R.M. Melampy. 1965. Oxytocin on ovarian function in cycling and hysterectomized heifers. J. Anim. Sci. 24:964-968.

Anderson, R.N., F.L. Schwartz and L.C. Ulberg. 1974. Adenylate cyclase activity of porcine corpora lutea. Biol. Reprod. 10:321-326.

Armstrong, D.T. and W. Hansel. 1959. Alteration of the bovine estrous cycle with oxytocin. J. Dairy Sci. 42:533-542.

Baird, D.T. 1977. Evidence *in vivo* for the two-cell hypothesis of oestrogen synthesis by the sheep Graafian follicle. J. Reprod. Fertil. 50:183-185.

Baird, D.T. 1978. Pulsatile secretion of LH and ovarian estradiol during the follicular phase of the sheep estrous cycle. Biol. Reprod. 18:359-364.

Baird, D.T., I.A. Swanston and A.S. McNeilly. 1981. Relationships between LH, FSH and prolactin concentrations and the secretion of androgens and estrogens by the preovulatory follicle of the ewe. Biol. Reprod. 24:1013-1025.

Bauminger, S., M.E. Lieberman and H.R. Lindner. 1975. Steroid-independent effect of gonadotropins on prostaglandin synthesis in rat Graafian follicles *in vitro*. Prostaglandins 9:753-764.

Bauminger, S., and H.R. Lindner. 1975. Preovulatory changes in ovarian prostaglandin formation and their hormonal control in the rat. Prostaglandins 9:737-751.

Beers, W.H. 1975. Follicular plasminogen and plasminogen activator and the effect of plasmin on ovarian follicle wall. Cell 6:379-386.

Beers, W.H., S. Strickland and E. Reich . 1975. Ovarian plasminogen activator: relationship to ovulation and hormonal regulation. Cell 6:387-394.

Behrman, H.R. 1979. Prostaglandins in hypothalamo-pituitary and ovarian function. Annu. Rev. Physiol. 41:685-700.

Behrman, H.R., J.L. Luborsky, R.F. Aten, M.L. Polan, B.C. Tarlatzis, F.P. Haseltine, S.L. Preston, L.K. Soodak, G.F. Mattson and A.S.Y. Chi. 1985. Luteolytic hormones are calcium-mediated, guanine nucleotide antagonists of gonadotropin-sensitive adenylate cyclase. Kyoto Conference on Prostaglandins p. 1-8.

Behrman, H.R, K. Yoshinga, H. Wyman and R.O. Greep. 1971. Effects of prostaglandins on ovarian steroid secretion and biosynthesis during pregnancy. Amer. J. Physiol. 221:189-193.

Benhaim, A., M. Herrou, H. Mittre and P. Leymarie. 1987 Effects of phorbol esters on steroidogenesis in small bovine luteal cells. FEBS Lett. 223:321-326.

Birnbaumer, L., J. Codina, R. Mattera, R.A. Cerione, J.D. Hildebrant, T. Sunyer, F.J. Rojas, M.G. Caron, R.J. Lefkowitz and R. Iyengar. 1985. Structural basis of adenylate cyclase stimulation and inhibition by distinct guanine nucleotide regulatory proteins. In: P. Cohen and M. Houslay (eds.) Molecular Mechanisms of Transmembrane Signaling. Elsevier. New York. pp. 131-182.

Bjersing, L., M.F. Hay, G. Kann, R.M. Moor, F. Naftolin, R.J. Scaramuzzi, R.V. Short and E.V. Younglai. 1972. Changes in gonadotrophins, ovarian steroids and follicular morphology in sheep at oestrus. J. Endocrinol. 52:465-479.

Blumberg, P.M. 1988. Protein kinase C as the receptor for the phorbol ester tumor promoters: sixth Rhoads memorial award lecture. Cancer Res. 48:1-8.

Braden, T.D., F. Gamboni and G.D. Niswender. 1988. Effects of prostaglandin $F_{2\alpha}$ -induced luteolysis on the populations of cells in the ovine corpus luteum. Biol. Reprod. 39:245-253.

Brunswig, B., A.K. Mukhopadhyay, L.T. Budnik, H.G. Bohnet and F.A. Leidenberger. 1986. Phorbol ester stimulates progesterone production by isolated bovine luteal cells. Endocrinology 118:743-749.

Burch, R.M., W.C. Wise, and P.V. Haluska. 1983. Prostaglandin-independent inhibition of calcium transport by nonsteroidal anti-inflammatory drugs: differential effects of carboxylic acids and piroxicam. J. Pharmacol. Exp. Therap. 227:84-91.Carson, R.S., J.K. Findlay, H.G. Burger, A.O. Trounson. 1979. Gonadotropin receptors of the ovine ovarian follicle during follicular growth and atresia. Biol. Reprod. 21:75-87.

Castagna, M., Y. Takai, K. Kaibuchi, K. Sano, U. Kikkawa and Y. Nishizuka. 1982. Direct activation of calcium-activated, phospholipid-dependent protein kinase by tumor-promoting phorbol ester. J. Biol. Chem. 257:7847-7851.

Channing, C.P. 1970. Effect of stage of the estrous cycle and gonadotrophins upon luteinization of porcine granulosa cells in culture. Endocrinology 87:156-164.

Channing, C.P. and S. Kammerman. 1973. Characteristics of gonadotropin receptors of porcine granulosa cells during follicle maturation. Endocrinology 92:531-540.

Chegini, N. and C.V. Rao. 1987. Dynamics of nuclear associated granules in bovine luteal cells after treatment in vitro with prostaglandin $F_{2\alpha}$. Endocrinology 121:1870-1878.

Chenault, J.R. 1983. Response of bovine corpora lutea to intrauterine prostaglandin E_2 infusion. J. Anim. Sci. 57(Suppl. 1):323.

Clark, M.R., J.M. Marsh, and W.J. LeMaire. 1978. Mechanism of luteinizing hormone regulation of prostaglandin synthesis in rat granulosa cells. J. Biol. Chem. 253:7757-7761.

Conn, P.M., M. Conti, J.P. Harwood, M.L. Dufau and K.J. Catt. 1978. Internalization of gonadotropin-receptor complex in ovarian luteal cells. Nature 274:598-600.

Cooke, R.G. and A.M. Homeida. 1982. Plasma concentrations of 13, 14-dihydro-15-keto prostaglandin $F_{2\alpha}$ and progesterone during oxytocin-induced estrous in the goat. Theriogenology 18:453-460.

Cooke, R.G. and A.M. Homeida. 1983. Prevention of the luteolytic action of oxytocin in the goat by inhibition of prostaglandin synthesis. Theriogenology 20:363-365.

Cooke, R.G. and A.M. Homeida. 1985. Suppression of prostaglandin $F_{2\alpha}$ release and delay of luteolysis after active immunization against oxytocin in the goat. J. Reprod. Fertil. 75:63-68.

Corner, G.W. 1961. The history of progesterone. In: A.C. Barnes (ed.) Brook Lodge Symposium. Progesterone. Brook Lodge Press. Augusta, Michigan. pp. 3-6.

Davis, J.S., H.W. Alila, L.A. West, R.A. Corradino and W. Hansel. 1988. Acute effects of prostaglandin $F_{2\alpha}$ on inositol phospholipid hydrolysis in the large and small cells of the bovine corpus luteum. Mol. Cell. Endocrinol. 58:43-50.

Davis, J.S., L.L. Weakland, R.V. Farese and L.A. West. 1987a. Luteinizing hormone increases inositol triphosphate and cytosolic free Ca^{2+} in isolated bovine luteal cells. J. Biol. Chem. 262:8515-8521.

Davis, J.S., L.L. Weakland, D.A. Weiland, R.V. Farese and L.A. West. 1987b. Prostaglandin $F_{2\alpha}$ stimulates phosphatidylinositol 4,5-bisphosphate hydrolysis and mobilizes intracellular Ca^{2+} in bovine luteal cells. Proc. Natl. Acad. Sci. USA 84:3728-3732.

Davis, J.S., L.L. Weakland, L.A. West and R.V. Farese. 1986a. Luteinizing hormone stimulates the formation of inositol triphosphate and cyclic AMP in rat granulosa cells. Evidence for phospholipase C generated second messengers in the action of luteinizing hormone. Biochem. J. 238:597-604.

Davis, J.S., L.A. West and R.V. Farese. 1984. Effects of luteinizing hormone on phosphoinositide metabolism in rat granulosa cells. J. Biol. Chem. 259:15028-15034.

Davis, J.S., L.A. West and R.V. Farese. 1986b. Gonadotropin-releasing hormone (GnRH) rapidly stimulates the formation of inositol phosphates and diacylglycerol in rat granulosa cells: further evidence for the involvement of Ca^{2+} and protein kinase C in the action of GnRH. Endocrinology 118:2561-2571.

Davis, J.S., L.A. West, L.L. Weakland and R.V. Farese. 1986c. Human chorionic gonadotropin activates the inositol 1,4,5-triphosphate-Ca^{2+} intracellular signalling system in bovine luteal cells. FEBS Lett. 208:287-291.

Diehl, J.R. and B.N. Day. 1974. Effect of prostaglandin $F_{2\alpha}$ on luteal function in swine. J. Anim. Sci. 39:392-396.

Diekman, M.A., P. O'Callaghan, T.M. Nett and G.D. Niswender. 1978. Effect of prostaglandin $F_{2\alpha}$ on the number of LH receptors in ovine corpora lutea. Biol. Reprod. 19:1010-1013.

Dieleman, S.J., Th.A.M. Kruip, P. Fontijne, W.H.R. de Jong and G.C. van der Weyden. 1983. Changes in oestradiol, progesterone and testosterone concentrations in follicular fluid and in the micromorphology of preovulatory bovine follicles relative to the peak of luteinizing hormone. J. Endocrinol. 97:31-42.

Dimino, M.J. and J. Snitzer. 1986. Luteinizing hormone (LH) causes rapid increases in accumulation of inositol phosphates in granulosa from medium follicles of porcine ovaries. Biol. Reprod. 34(Suppl. 1):168.

Donaldson, L.E., J.M. Bassett and G.D. Thorburn. 1970. Peripheral plasma progesterone concentration of cows during puberty, oestrous cycles, pregnancy and lactation, and the effects of under-nutrition or exogenous oxytocin on progesterone concentration. J. Endocrinol. 48:599-614.

Donaldson, L.E. and W. Hansel. 1965. Prolongation of the life span of the bovine corpus luteum by single injections of bovine luteinizing hormone. J. Dairy Sci. 48:903-904.

Donaldson, L.E., W. Hansel and L.D. VanVleck. 1965. The luteotropic properties of luteinizing hormone and the nature of oxytocin induced luteal inhibition in cattle. J. Dairy. Sci. 48:331-337.

Dorflinger, L.J., P.J. Albert, A.T. Williams and H.R. Behrman. 1984. Calcium is an inhibitor of luteinizing hormone-sensitive adenylate cyclase in the luteal cell. Endocrinology 114:1208-1215.

Dorflinger, L.J., J.L. Luborsky, S.D. Gore and H.R. Behrman. 1983. Inhibitory characteristics of prostaglandin $F_{2\alpha}$ in the rat luteal cell. Mol. Cell. Endocrinol. 33:225-241.

Dorrington, J.H., Y.S. Moon and D.T. Armstrong. 1975. Estradiol-17ß biosynthesis in cultured granulosa cells from hypophysectomized immature rats; stimulation by follicle-stimulating hormone. Endocrinology 97:1328-1331.

Downes, P. and R. Michell. 1985. Inositol phospholipid breakdown as a receptor controlled generator of second messengers. In: P. Cohen & M.D. Houslay (eds.). Molecular Aspects of Cellular Regulation, Vol. 4. Molecular Mechanisms of Transmembrane Signaling. Elsevier, Amsterdam pp. 3-56.

Duncan, G.W., A.M. Bowerman, L.L. Anderson, W.R. Hearn and R.M. Melampy. 1961. Factors influencing in vitro synthesis of progesterone. Endocrinology 68:199-207.

Dunlap, K., G.G. Holz, and S.G. Rane. 1987. G proteins as regulators of ion channel function. Trends Neurosci. 10:241-244.

England, B.G., M.K. Dahmer and R. Webb. 1981a. Relationship between follicular size and antral fluid steroid concentrations at three stages of the estrous cycle in the ewe. Biol. Reprod. 24:1068-1075.

England, B.G., R. Webb and M.K. Dahmer. 1981b. Follicular steroidogenesis and gonadotropin binding to ovine follicles during the estrous cycle. Endocrinology 109:881-887.

Erickson, G.F., J.R.G. Challis and K.J. Ryan. 1977. Production of prostaglandin F by rabbit granulosa cells and thecal tissue. J. Reprod. Fertil. 49:133-134.

Espey, L.L. 1971. Decomposition of connective tissue in rabbit ovarian follicles by multivesicular structures of thecal fibroblasts. Endocrinology 88:437-444.

Espey, L.L. 1978. Ovarian contractility and its relationship to ovulation: a review. Biol. Reprod. 19:540-541.

Espey, L.L. 1980. Ovulation as an inflammatory reaction- a hypothesis. Biol. Reprod. 22:73-106.

Espey, L.L. and P. Rondell. 1968. Collagenolytic activity in the rabbit and sow Graafian follicle during ovulation. Amer. J. Physiol. 214:326-329.

Evans, G., M. Dobias, G.J. King and D.T. Armstong. 1981. Estrogen, androgen and progesterone biosynthesis by theca and granulosa of preovulatory follicles in the pig. Biol. Reprod. 25:673-682.

Fairclough, R.J., J.F. Smith and L.T. McGowan. 1981. Prolongation of the oestrous cycle in cows and ewes after passive immunization with PGF antibodies. J. Reprod. Fertil. 62:213-219.

Farin, C.E., C.L. Moeller, H.R. Sawyer, F. Gamboni and G.D. Niswender. 1986. Morphometric analysis of cell types in the ovine corpus luteum throughout the estrous cycle. Biol. Reprod. 35:1299-1308.

Fehr, S., R. Ivell, R. Koll, D. Schams, M. Fields and D. Richter. 1987. Expression of the oxytocin gene in the large cells of the bovine corpus luteum. 210:45-50.

Fields, P.A., R.K. Eldridge, A.R. Fuchs, R.F. Roberts and M.J. Fields. 1983. Human placental and bovine corpora luteal oxytocin. Endocrinology 112:1544-1546.

Fields, M.J. and P.A. Fields. 1986. Luteal neurophysin in the nonpregnant cow and ewe: immunocytochemical localization in membrane-bounded secretory granules of the large luteal cell. Endocrinology 118:1723-1725.

Fitz, T.A., J.L. Fleeger, M.F. Smith and P.G. Harms. 1980. Human chorionic gonadotropin (hCG) binding and adenylate cyclase (AC) activity in developing and regressing bovine corpora lutea (CL). Biol. Reprod. 22(Suppl. 1):61a.

Fitz, T.A., M.H. Mayan, H.R. Sawyer and G.D. Niswender. 1982. Characterization of two steroidogenic cell types in the ovine corpus luteum. Biol. Reprod. 27:703-711.

Fitz, T.A., E.J. Mock, M.H. Mayan and G.D. Niswender. 1984a. Interactions of prostaglandins with subpopulations of ovine luteal cells. I. Stimulatory effects of prostaglandins E_1, E_2, and I_2. Prostaglandins 28:119-126.

Fitz, T.A., E.J. Mock, M.H. Mayan and G.D. Niswender. 1984b. Interactions of prostaglandins with subpopulations of ovine luteal cells. II. Inhibitory effects of $PGF_{2\alpha}$ and protection by PGE_2. Prostaglandins 28:127-138.

Fletcher, P.W. and G.D. Niswender. 1982. Effect of $PGF_{2\alpha}$ on progesterone secretion and adenylate cyclase activity in ovine luteal tissue. Prostaglandins 23:803-818.

Flint, A.P.F. and E.L. Sheldrick. 1982a. Ovarian secretion of oxytocin in the sheep. J. Physiol. (Lond.) 330:61P-62P.

Flint, A.P.F. and E.L. Sheldrick. 1982b. Ovarian secretion of oxytocin is stimulated by prostaglandin. Nature 297:587-588.

Flint, A.P.F. and E.L. Sheldrick. 1985. Continuous infusion of oxytocin prevents induction of uterine oxytocin receptor and blocks luteal regression in cyclic ewes. J. Reprod. Fertil. 75:623-631.

Flower, R.J. 1974. Drugs which inhibit prostaglandin biosynthesis. Pharmacol. Rev. 26:33-67.

Fortune, J.E. and D.T. Armstrong. 1978. Hormonal control of 17ß-estradiol biosynthesis in proestrous rat follicles: estradiol production by isolated theca versus granulosa. Endocrinology 102:227-235.

Geenan, V., J.J. Legros, M.T. Hazee-Hagelstein, F. Louis-Kohn, M.J. Lecomte-Yerna, A. Demoulin and P. Franchimont. 1985. Release of immunoreactive oxytocin and neurophysin I by cultured luteinizing bovine granulosa cells. Acta Endocrinol. 110:263-270.

Gengenbach, D.R., J.E. Hixon and W. Hansel. 1977. A luteolytic interaction between estradiol and prostaglandin $F_{2\alpha}$ in hysterectomized ewes. Biol. Reprod. 16:571-579.

Gilbert, C.L., A.P.F. Flint, G.E. Lamming, T.J. Parkinson and D.C. Wathes. 1987. Oxytocin (OXT) infusion from day 10 after oestrus extends the luteal phase in non-pregnant cattle. Soc. Study Fertil. Winter Meeting. Programme and Abstracts of Papers. p. 22.

Gilman, A.G. 1987. G proteins: transducers of receptor-generated signals. Annu. Rev. Biochem. 56:615-649.

Ginther, O.J., C.O. Woody, S. Mahajan, K. Janakiraman and L.E. Casida. 1967. Effect of oxytocin administration on the oestrous cycle of unilaterally hysterectomized heifers. J. Reprod. Fertil. 14:225-229.

Goldenberg R.L., J.L. Vaitukaitis and G.T. Ross. 1972. Estrogen and follicle stimulating hormone interactions on follicle growth in rats. Endocrinology 90:1492-1498.

Goldring, N.B., J.M. Durica, J. Lifka, L. Hedin, S.L. Ratoosh, W.L. Miller, J. Orley and J.S. Richards. 1987. Cholesterol side-chain cleavage P450 messenger ribonucleic acid: evidence for hormonal regulation in rat ovarian follicles and constitutive expression in corpora lutea. Endocrinology 120:1942-1950.

Goodman A.L. and G.D. Hodgen. 1983. The ovarian triad of the primate menstrual cycle. Rec. Prog. Horm. Res. 39:1-73.

Guldenaar, S.E.F., D.C. Wathes and B.T. Pickering. 1984. Immunocytochemical evidence for the presence of oxytocin and neurophysin in the large luteal cells of the bovine corpus luteum. Cell Tiss. Res. 237:349-352.

Hafez, E.S.E., M-C Lavasseur and C. Thibault. 1980. Folliculogenesis, egg maturation, and ovulation. In:E.S.E. Hafez (ed.) Reproduction in Farm Animals. Lea and Febiger. Philadelphia. pp.150-166.

Hansel, W., P.W. Concannon and J.H. Lukaszewska. 1973. Corpora lutea of the large domestic animals. Biol. Reprod. 8:222-245.

Hansel, W. and E.M. Convey. 1983. Physiology of the estrous cycle. J. Anim. Sci. 57(Suppl. 2):404-424.

Hansel, W. and W.C. Wagner. 1960. Luteal inhibition in the bovine as a result of oxytocin injection, uterine dilatation, and intrauterine infusion of seminal and preputial fluids. J. Dairy Sci. 43:796-805.

Harrison, L.M., N. Kenny and G.D. Niswender. 1987. Progesterone production, LH receptors, and oxytocin secretion by ovine luteal cell types on days 6, 10 and 15 of the oestrus cycle and day 25 of pregnancy. J. Reprod. Fertil. 79:539-548.

Henderson, K.M., R.J. Scaramuzzi and D.T. Baird. 1977. Simultaneous infusion of prostaglandin E_2 antagonizes the luteolytic action of prostaglandin $F_{2\alpha}$ in vivo. J. Endocrinol. 72:379-383.

Hillier, S.G. 1987. Paracrine control of the ovaries. Res. Reprod. 19:1-2.

Hirst, J.J., G.E. Rice, G. Jenkin and G.D. Thorburn. 1986. Secretion of oxytocin and progesterone by ovine corpora lutea in vitro. Biol. Reprod. 35:1106-1114.

Hirst, J.J., G.E. Rice, G. Jenkin and G.D. Thorburn. 1988. Control of oxytocin secretion by ovine corpora lutea: effects of arachidonic acid, phospholipases, and prostaglandins. Endocrinology 122:774-781.

Hisaw, F.L. 1947. Development of the Graafian folllicle and ovulation. Physiol. Rev. 27:95-119.

Homeida, A.M. 1986. Evidence for the presence of oxytocin in the corpus luteum of the goat. Br. J. Pharmacol. 87:673-677.

Homeida, A.M. and A.E. Khalafalla. 1987. Effects of oxytocin-antagonist injections on luteal regression in the goat. Br. J. Pharmacol. 90:281-284.

Hoyer, P.B., T.A. Fitz and G.D. Niswender. 1984. Hormone-independent activation of adenylate cyclase in large steroidogenic ovine luteal cells does not result in increased progesterone secretion. Endocrinology 114:604-608.

Hutchison J.S. and A.J. Zeleznik. 1984. The rhesus monkey corpus luteum is dependent upon pituitary gonadotropin secretion throughout the luteal phase of the menstrual cycle. Endocrinology 115:1780-1786.

Ireland, J.J. 1987. Control of follicular growth and development. J. Reprod. Fertil. 34(Suppl):39-54.

Ireland, J.J., P.B. Coulson and R.L. Murphree. 1979. Follicular development during four stages of the estrous cycle of beef cattle. J. Anim. Sci. 49:1261-1269.

Ivell, R., K.H. Brackett, M.J. Fields, and D. Richter. 1985. Ovulation triggers oxytocin gene expression in the bovine ovary. FEBS Lett. 190:263-267.

Ivell, R. and D. Richter. 1984. The gene for the hypothalamic peptide hormone oxytocin is highly expressed in the bovine corpus luteum: biosynthesis, structure and sequence analysis. EMBO J. 3:2351-2354.

Jacobson, L., R.K. Riemer, A.C. Goldfien, D. Lykins, P.K. Siiteri and J.M.Roberts. 1987. Rabbit myometrial oxytocin and alpha sub(2)-adrenergic receptors are increased by estrogen but are differentially regulated by progesterone. Endocrinology 120:1184-1188.

Jungclas, B. and M.R. Luck. 1986. Evidence for granulosa-theca interaction in the secretion of oxytocin by bovine ovarian tissue. J. Endocrinol. 109:R1-R4.

Kaibuchi, K., Y. Takai, M. Sawamura, T. Fujikura and Y. Nishizuka. 1983. Synergistic functions of protein phosphorylation and calcium mobilization in platelet activation. J. Biol. Chem. 258:6701-6707.

Kaltenbach, C.C., J.W. Graber, G.D. Niswender and A.V. Nalbandov. 1968a. Effect of hypophysectomy on the formation and maintenance of corpora lutea in the ewe. Endocrinology 82:753-759.

Kaltenbach, C.C., J.W. Graber, G.D. Niswender and A.V. Nalbandov. 1968b. Luteotrophic properties of some pituitary hormones in non-pregnant or pregnant hypophysectomized ewes. Endocrinology 82:818-824.

Karsch, F.J., B. Cook, A.R. Ellicott, P.L. Foster, G.L. Jackson and A.V. Nalbandov. 1971. Failure of infused prolactin to prolong the life span of the corpus luteum of the ewe. Endocrinology 89:272-275.

Kawai, Y. and M.R. Clark. 1985. Phorbol ester regulation of rat granulosa cell prostaglandin and progesterone accumulation. Endocrinology 116:2320-2326.

Kesner, J.S., E.M. Convey and C.R. Anderson. 1981. Evidence that estradiol induces the preovulatory LH surge in cattle by increasing pituitary sensitivity to LHRH and then increasing LHRH release. Endocrinology 108:1386-1391.

Kidwell, W.R., K. Barlogh, Jr. and W.G. Wiest. 1966. Effects of luteinizing hormones on glucose-6-phosphate and 20α -hydroxysteroid dehydrogenase activities in superovulated rat ovaries. Endocrinology 79:352-361.

Koligian, K.B. and F. Stormshak. 1977. Nuclear and cytoplasmic estrogen receptors in ovine endometrium during the estrous cycle. Endocrinology 101:524-533.

Kraeling, R.R., C.R. Barb and B.J. Davis. 1975. Prostaglandin-induced regression of porcine corpora lutea maintained by estrogen. Prostaglandins 9:459-462.

Kruip, T.A.M., H.G.B. Vullings, D. Schams, J. Jonis and A. Klarenbeek. 1985. Immunocytochemical demonstration of oxytocin in bovine ovarian tissues. Acta Endocrinol. 109:537-542.

Lang, D.R. 1965. Synchronization of the ovarian cycle of the ewe after surgical suppression of the corpus luteum. J. Reprod. Fertil. 9:113-114.

LeMaire, W.J. and J.M. Marsh. 1975. Interrelationships between prostaglandins, cyclic AMP and steroids in ovulation. J. Reprod. Fertil. 22(Suppl.):53-74.

LeMaire, W.J., N.S.T. Yang, H.H. Behrman and J.M. Marsh. 1973. Preovulatory changes in the concentration of prostaglandins in the rabbit Graafian follicles. Prostaglandins 3:367-376.

Lemon, M., M. Loir. 1977. Steroid release in vitro by two luteal cell types in the corpus luteum of the pregnant sow. J. Endocrinol. 72:351-59.

Leung, P.C.K., T. Minegishi, F. Ma, F. Zhou and B. Ho-Yuen. 1986. Induction of polyphosphoinositide breakdown in rat corpus luteum by prostaglandin $F_{2\alpha}$. Endocrinology 119:12-18.

Lindner, H.R., A. Amsterdam, Y. Solomon, A. Tsafriri, A. Nimrod, S.A. Lamprecht, U. Zor, Y. Koch. 1977. Intraovarian factors in ovulation: determinants of follicular response to gonadotropins. J. Reprod. Fertil. 51:215-235.

Lipner, H. and R.O. Greep. 1971. Inhibition of steroidogenesis at various sites in the biosynthetic pathway in relation to induced ovulation. Endocrinology 88:602-607.

Loeb, L. 1927. The effects of hysterectomy on the system of sex organs and on their periodicity of the sexual cycle in the guinea pig. Amer. J. Physiol. 83:202.

Luborsky, J.L., L.J. Dorflinger, K. Wright and H.R. Behrman. 1984a. Prostaglandin $F_{2\alpha}$ inhibits luteinizing hormone (LH)-induced increase in LH receptor binding to isolated rat luteal cells. Endocrinology 115:2210-2216.

Luborsky, J.L., W.T. Slater and H.R. Behrman. 1984b. Luteinizing hormone aggregation by prostaglandin $F_{2\alpha}$ and ferritin-LH. Endocrinology 115:2217-2226.

Lukaszewska, J.H., L. Wilson and W. Hansel. 1972. Luteotropic and luteolytic effects of prostaglandins in the hamster. Proc. Soc. Exp. Biol. Med. 140:1302-1307.

Ma, F. and P.K.C. Leung. 1985. Luteinizing hormone-releasing hormone enhances polyphosphoinositide breakdown in rat granulosa cells. Biochem. Biophys. Res. Comm. 130:1201-1208.

Mais, V., R.R. Kazer, N.S. Cetel, J. Rivier, W. Vale and S.S.C. Yen. 1986. The dependency of folliculogenesis and corpus luteum function on pulsatile gonadotropin secretion in cycling women using a gonadotropin-releasing hormone antagonist as a probe. J. Clin. Endocrinol. Metab. 62:1250-1255.

Majerus, P.W., T.M. Connolly, H. Deckmyn, T.S. Ross, T.E. Bross, H. Ishii, V.S. Bansal and D.B. Wilson. 1986. The metabolism of phosphoinositide-derived messenger molecules. Science 234:1519-1526.

Marsh, J.M., R.W. Butcher, K. Savard and E.W. Sutherland. 1966. The stimulatory effect of luteinizing hormone on adenosine 3'-5'-monophosphate accumulation in corpus luteum slices. J. Biol. Chem. 241:5436-5440.

Marsh, J.M. and W.J. Lemaire. 1973. The role of cyclic AMP and prostaglandins in the actions of luteinizing hormone. In: N.R. Moudgal (ed). Gonadotropins and Gonadal Function. Academic Press, New York. pp. 376-390.

Marsh, J.M., T.M. Mills and W.J. LeMaire. 1973. Preovulatory changes in the synthesis of cyclic AMP by rabbit Graafian follicles. Biochim. Biophys. Acta 304:197-202.

Mason, N.R., J.M. Marsh and K. Savard. 1962. An action of gonadotropin in vitro. J. Biol. Chem. 237:1801-06.

Matton, P., V. Adelakoun, Y. Couture and J.J. Dufour. 1981. Growth and replacement of the bovine ovarian follicles during the estrous cycle. J. Anim. Sci. 52:813-820.

McCann, T.J. and A.P.F. Flint. 1987. Prostaglandin $F_{2\alpha}$, cyclic adenosine monophosphate and inositol triphosphate in sheep corpus luteum. Biol. Reprod. 35(Suppl. 1):163.

McCracken, J.A., W. Schramm and W.C. Okulicz. 1984. Hormone receptor control of pulsatile secretion of $PGF_{2\alpha}$ from the ovine uterus during luteolysis and its abbrogation in early pregnancy. Anim. Reprod. Sci. 7:31-55.

McNatty, K.P., D.A. Heath, K.M. Henderson, S. Lun, P.R. Hurst, L.M. Ellis, G.W. Montgomery, L. Morrison and D.C. Thurley. 1984. Some aspects of thecal and granulosa cell function during follicular development in the bovine ovary. J. Reprod. Fertil. 72:39-53.

Milne, J.A. 1963. Effects of oxytocin on the oestrous cycle of the ewe. Austr. Vet. J. 39:51-52.

Milvae, R.A. and W. Hansel. 1980. Concurrent uterine venous and ovarian arterial prostaglandin F concentrations in heifers treated with oxytocin. J. Reprod. Fertil. 60:7-15.

Mitchell, M.D., A.P.F. Flint and A.C. Turnbull. 1975. Stimulation by oxytocin of prostaglandin F levels in uterine venous effluent in pregnant and puerperal sheep. Prostaglandins 9:47-56.

Mitchell, M.D., D.L. Kraemer, S.P. Brennecke and R. Webb. 1982. Pulsatile release of oxytocin during the estrous cycle, pregnancy and parturition in sheep. Biol. Reprod. 27:1169-1173.

Moeljono, M.P.E., F.W. Bazer and W.W. Thatcher. 1976. A study of prostaglandin $F_{2\alpha}$ as the luteolysin in swine. I. Effect of prostaglandin $F_{2\alpha}$ in hysterectomized gilts. Prostaglandins 11:737-743.

Moon, Y.S., J.H. Dorrington and D.T. Armstrong. 1975. Stimulatory action of follicle-stimulating hormone on estradiol-17ß secretion by hypophysectomized rat ovaries in organ culture. Endocrinology 97:244-247.

Moor, R.M. 1974. The ovarian follicle of the sheep: inhibition of oestrogen secretion by luteinizing hormone. J. Endocrinol. 61:455-463.

Moor, R.M. 1977. Sites of steroid production in ovarian Graafian follicles in culture. J. Endocrinol. 73:143-150.

Moor, R.M., M.F. Hay, H.M. Dott and D.G. Cran. 1978. Macroscopic identification and steroidogenic function of atretic follicles in sheep. J. Endocrinol. 77:309-318.

Moor, R.M., M.F. Hay and R.F. Seamark. 1975. The sheep ovary: regulation of steroidogenic, haemodynamic and structural changes in the largest follicle and adjacent tissue before ovulation. J.Reprod. Fertil. 45:595-604.

Moore, J.J., G.R. Dubyak, R.M. Moore and D. Vander Kooy. 1988. Oxytocin activates the inositol-phospholipid-protein kinase-C system and stimulates prostaglandin production in human amnion cells. Endocrinology 123:1771-1777.

Nakano, R., T. Akahori, K. Katayama and S. Tojo. 1977. Binding of LH and FSH to porcine granulosa cells during follicular maturation. J. Reprod. Fertil. 51:23-27.

Nishizuka, Y. 1986. Studies and perspectives of protein kinase C. Science. 233:305-312.

Nishizuka, Y., Y. Takai, A. Kishimoto, U. Kikkawa and K. Kaibuchi. 1984. Phospholipid turnover in hormone action. Rec. Prog. Horm. Res. 40:301-345.

Northover, A.M. 1982. A study of the effects of indomethacin, flufenamate and salicylate on localization of intracellular calcium in rabbit neutrophil polymorphs using an antimonate staining method. Br. J. Exp. Pathol. 63:686-692.

Northover, A.M. 1985. The effects of some non-steroidal anti-inflammatory agents on membrane-associated calcium in rabbit peritoneal neutrophils. Biochem. Pharmacol. 34:3123-3129.

Orczyk, G.P. and H.R. Behrman. 1972. Ovulation blockade by aspirin or indomethacin *in vivo* evidence for a role of prostaglandin in gonadotropin secretion. Prostaglandins 1:3-20.

O'Shea, J.D. 1971. Smooth muscle-like cells in the theca externa of ovarian follicles in sheep. J. Reprod. Fertil. 24:283-285.

O'Shea, J.D. and R.E. Phillips.1974. Contractility in vitro of ovarian follicles from sheep and the effects of drugs. Biol Reprod. 10:370-379.

Oyedipe, E.O., B. Gustafsson and H. Kindahl. 1984. Blood levels of progesterone and 15-keto-13, 14-dihydro prostaglandin $F_{2\alpha}$ during the estrous cycle of oxytocin-treated cows. Theriogenology 22:329-339.

Padmanabhan, V., E.M. Convey, J.F. Roche and J.J. Ireland. 1984. Changes in inhibin-like bioactivity in ovulatory and atretic follicles and utero-ovarian venous blood after prostaglandin-induced luteolysis in heifers. Endocrinology 115:1332-1340.

Parr, E.L. 1974. Histological examination of the rat ovarian follicle wall prior to ovulation. Biol. Reprod. 11:483-503.

Pencharz, R.I. 1940. Effect of estrogens and androgens alone and in combination with chorionic gonadotropin on the ovary of the hypophysectomized rat. Science 91:554-555.

Pierce, J.G. and T.F. Parsons. 1981. Glycoprotein hormones: structure and function. Annu. Rev. Biochem. 50:465-495.

Pineda, M.H., M.C. Garcia and O.J. Ginther. 1973. Effect of antiserum against an equine pituitary fraction on corpus luteum and follicles in mares during diestrus. Amer. J. Vet. Res. 34:181-183.

Pool, W.R. and H. Lipner. 1966. Inhibition of ovulation by antibiotics. Endocrinology 79:858-864.

Pratt, B.R., R.L. Butcher and E.K. Inskeep. 1977. Antiluteolytic effect of the conceptus and of PGE_2 in ewes J. Anim. Sci. 45:784-791.

Pupkin, M., H. Bratt, J. Weisz, C.W. Lloyd and K. Balogh, Jr. 1966. Dehydrogenases in the rat ovary. I. A histochemical study of Δ^5-3ß- and 20α -hydroxysteroid dehydrogenases and enzymes of carbohydrate oxidation during the estrous cycle. Endocrinology 79:352-361.

Rajaniemi, H.J., A.R. Midgley, Jr., J.A. Duncan and L.E. Reichert. 1977. Gonadotropin receptors in rat ovarian tissue: III. Binding sites for luteinizing hormone and differentiation of granulosa cells to luteal cells. Endocrinology 101:898-910.

Rao, M.C., A.R. Midgley, Jr. and J.S. Richards. 1978. Hormonal regulation of ovarian cellular proliferation. Cell 14:71-78.

Raymond, V., P.C.K. Leung and F. Labrie. 1983. Stimulation by prostaglandin $F_{2\alpha}$ of phosphatidic acid-phosphatidylinositol turnover in rat luteal cells. Biochem. Biophys. Res. Comm. 116:39-46.

Rice, G.E. and G.D. Thorburn. 1985. Subcellular localization of oxytocin in the ovine corpus luteum. Can. J. Physiol. Pharmacol. 63:309-314.

Richards, J.S. 1975. Estradiol receptor content in rat granulosa cells during follicular development: modification by estradiol and gonadotropins. Endocrinology 97:1174-1184.

Richards, J.S. 1980. Maturation of ovarian follicles: actions and interactions of pituitary and ovarian hormones on follicular cell differentiation. Physiol. Rev. 60:51-89.

Richards, J.S. and K. Bogovich. 1982. Effects of human chorionic gonadotropin and progesterone on follicular development in the immature rat. Endocrinology 111:1429-1438.

Richards, J.S., J.J. Ireland, M.C. Rao, G.A. Bernath, A.R. Midgley, Jr. and L.E. Reichert, Jr. 1976. Ovarian follicular development in the rat: hormone receptor regulation by estradiol, follicle stimulating hormone and luteinizing hormone. Endocrinology 99:1562-1570.

Richards, J.S., T. Jahnsen, L. Hedin, J. Lifka, S. Ratoosh, J.M. Durica and N.B. Goldring. 1987. Ovarian follicular development: from physiology to molecular biology. Rec. Prog. Horm. Res. 43:231-276.

Roberts, J.S., B. Barcikowski, L.Wilson, R.C. Skarnes and J.A. McCracken. 1975. Hormonal and related factors affecting the release of prostaglandin $F_{2\alpha}$ from the uterus. J. Ster. Biochem. 6:1091-1097.

Roberts, J.S., J.A. McCracken, J.E. Gavagan and M.S. Soloff. 1976. Oxytocin-stimulated release of prostaglandin $F_{2\alpha}$ from ovine endometrium in vivo: correlation with estrous-cycle and oxytocin-receptor binding. Endocrinology 99:1107-1114.

Roberts, J.S. and L. Share. 1969. Effects of progesterone and estrogen on blood levels of oxytocin during vaginal distention. Endocrinology 84:1076-1081.

Rodgers, R.J., J.I. Mason, M.R. Waterman and E.R. Simpson. 1987a. Regulation of the synthesis of 3-hydroxy-3-methylglutaryl coenzyme A reductase in the bovine ovary *in vivo* and *in vitro*. Mol. Endocrinol. 1:172-180.

Rodgers, R.J. and J.D. O'Shea. 1982. Purification, morphology, and progesterone production and content of three cell types isolated from the corpus luteum of the sheep. Austr. J. Biol. Sci. 35:441-455.

Rodgers, R.J., J.D. O'Shea and J.K. Findlay. 1985. Do small and large luteal cells of the sheep interact in the production of progesterone? J. Reprod. Fertil. 75:85-94.

Rodgers, R.J., J.D. O'Shea, J.K. Findlay, A.P.F. Flint and E.L. Sheldrick. 1983. Large luteal cells the source of luteal oxytocin in the sheep. Endocrinology 113:2302-2304.

Rodgers, R.J., H.F. Rodgers, P.F. Hall, M.R. Waterman and E.R. Simpson. 1986a. Immunolocalization of cholesterol side-chain-cleavage cytochrome P-450 and 17α -hydroxylase cytochrome P-450 in bovine ovarian follicles. J. Reprod. Fertil. 78:627-638.

Rodgers, R.J., H.F. Rodgers, M.R. Waterman and E.R. Simpson. 1986b. Immunolocalization of cholesterol side-chain-cleavage cytochromeP-450 and ultrastructural studies of bovine corpora lutea. J. Reprod. Fertil. 78:639-652.

Rodgers, R.J., H.F. Rodgers, M.R. Waterman and E.R. Simpson. 1986c. Cytochromes $P\text{-}450_{scc}$, $P\text{-}450_{17\alpha}$, adrenodoxin, and reduced nicotinamide adenine dinucleotide phosphate-cyctochrome P-450 reductase in bovine follicles and corpora lutea. Changes in specific contents during the ovarian cycle. Endocrinology 118:1366-1374.

Rodgers, R.J., M.R. Waterman and E.R. Simpson. 1987b. Levels of messenger ribonucleic acid encoding cholesterol side-chain cleavage cytochrome P-450, 17_{α} -hydroxylase cytochrome P-450, adrenodoxin and low density lipoprotein receptor in bovine follicles and corpora lutea throughout the estrous cycle. Mol. Endocrinol. 1:274-279.

Roser, J.F. and J.W. Evans. 1983. Luteal luteinizing hormone receptors during the postovulatory period in the mare. Biol. Reprod. 29:499-510.

Sawyer, H.R., C.L. Moeller and G.P. Kozlowski. 1986. Immunocytochemical localization of neurophysin and oxytocin in ovine corpora lutea. Biol. Reprod. 34:543-548.

Schallenberger, E., D. Schams, B. Bullermann and D.L. Walters. 1984. Pulsatile secretion of gonadotrophins, ovarian steroids and ovarian oxytocin during prostaglandin-induced regression of the corpus luteum in the cow. J. Reprod. Fertil. 71:493-501.

Schams, D. 1983. Oxytocin determination by radioimmunoassay. III. Improvement to subpicogram sensitivity and application to blood levels in cyclic cattle. Acta Endocrinol. 103:180-183.

Schams, D. and A. Lahlou-Kassi. 1984. Circulating concentrations of oxytocin during pregnancy in ewes. Acta Endocrinol. 106:277-281.

Schams, D., A. Lahlou-Kassi and P. Glatzel. 1982. Oxytocin concentrations in peripheral blood during the estrous cycle and after ovariectomy in two breeds of sheep with low and high fecundity. J. Endocrinol. 92:9-13.

Schams, D., S. Prokopp and D. Barth. 1983. The effect of active and passive immunization against oxytocin on ovarian cyclicity in ewes. Acta Endocrinol. 103:337-344.

Schams, D., E. Schallenberger and J.J. Legros. 1985. Evidence for the secretion of immunoreactive neurophysin I in addition to oxytocin from the ovary in cattle. J. Reprod. Fertil. 73:165-171.

Schramm, W., L. Bovaird, M.E. Glew, G. Schramm and J.A. McCracken. 1983. Corpus luteum regression induced by ultra-low pulses of prostaglandin $F_{2\alpha}$. Prostaglandins 26:347-364.

Schwall, R.H., F. Gamboni, M.H. Mayan and G.D. Niswender. 1986. Changes in the distribution of sizes of ovine luteal cells during the estrous cycle. Biol. Reprod. 34:911-918.

Seamark, R.F., R.M. Moor and J.E.A. McIntosh. 1974. Steroid hormone production by sheep ovarian follicles cultured *in vitro*. J. Reprod. Fertil. 41:143-158.

Sellner, R.G. and E.W. Wickersham. 1970. Effects of prostaglandins on steroidogenesis. J. Anim. Sci. 31:230.

Sharma, S.C. and R.J. Fitzpatrick. 1974. Effects of oestradiol-17ß and oxytocin treatment on prostaglandin F alpha release in the anoestrous ewe. Prostaglandins 6:97-105.

Sheldrick, E.L. and A.P.F. Flint. 1981. Circulating concentrations of oxytocin during the estrous cycle and early pregnancy in sheep. Prostaglandins 22:631-636.

Sheldrick, E.L. and A.P.F. Flint. 1983a. Regression of the corpora lutea in sheep in response to chloprostenol is not affected by loss of luteal oxytocin after hysterectomy. J. Reprod. Fertil. 68:155-160.

Sheldrick, E.L. and A.P.F. Flint. 1983b. Luteal concentrations of oxytocin decline during early pregnancy in the ewe. J. Reprod. Fertil. 68:477-480.

Sheldrick, E.L. and A.P.F. Flint. 1985a. Endocrine control of uterine oxytocin receptors in the ewe. J. Endocrinol. 106:249-258.

Sheldrick, E.L. and A.P.F. Flint. 1985b. Luteal oxytocin and the control of prostaglandin-$F_{2\alpha}$ secretion during luteolysis in ewes. J. Endocrinol. 107(Suppl.) No. 89.

Sheldrick, E.L. and A.P.F. Flint. 1987. Continuous infusion of oxytocin delays luteolysis in the ewe by reducing prostaglandin $F_{2\alpha}$ secretion. Soc. Study Reprod. Programme and Abstracts of Papers. p.22.

Sheldrick, E.L., M.D. Mitchell and A.P.F. Flint. 1980. Delayed luteal regression in ewes immunized against oxytocin. J. Reprod. Fertil. 59:37-42.

Shemesh, M., J.F. Strauss III and W. Hansel. 1985. The role of calcium and protein kinase C in the control of bovine placental progesterone synthesis. Biol. Reprod. 32(Suppl. 1): 54.

Smith, W.L. and W.E.M. Lands. 1971. Stimulation and blockade of prostaglandin biosynthesis. J. Biol. Chem. 246, 6700-6704.

Speroff L. and P.W. Ramwell. 1970. Prostaglandin stimulation of *in vitro* progesterone synthesis. J. Clin. Endocrinol. Metab. 30:345-350.

Spicer, L.J., J.J. Ireland and J.F. Roche. 1981. Changes in serum LH, progesterone, and specific binding of ^{125}I-hCG to luteal cells during regression and development of bovine corpora lutea. Biol. Reprod. 25:832-841.

Staigmiller, R.B., B.G. England, R. Webb, R.E. Short and R.A. Bellows. 1982. Estrogen secretion and gonadotropin binding by individual bovine follicles during estrus. J. Anim. Sci. 55:1473-1482.

Stormshak, F., M.B. Zelinski-Wooten and S.E. Abdelgadir. 1987. Comparative aspects of the regulation of corpus luteum function in various species. In: V.B. Mahesh and D.S. Dhindsa, E. Anderson and S.P. Kalra (eds.). Regulation of Ovarian and Testicular Function. Plenum Publ. Co. New York.

Strickland, S. and W.H. Beers. 1976. Studies on the role of plasminogen activator in ovulation. In vitro response of granulosa cells to gonadotropins, cyclic nucleotides, and prostaglandins. J. Biol. Chem. 251:5694-5702.

Swann, R.W., P.L. O'Shaughnessy, S.D. Birkett, D.C. Wathes, D.G. Porter, and P.T. Pickering. 1984. Biosynthesis of oxytocin in the corpus luteum. FEBS Lett. 174:262-266.

Tan, G.J.S. and J.S.G. Biggs. 1984. Progesterone production by dispersed luteal cells of non-pregnant cows: effects of oxytocin and oestradiol. Anim. Reprod. Sci. 7:441-445.

Tan, G.J.S., R. Tweedale and J.S.G. Biggs. 1982. Effect of oxytocin on the bovine corpus luteum of early pregnancy. J. Reprod. Fertil. 66:75-78.

Theodosis, D.T., F.B.P. Wooding, E.L. Sheldrick and A.P.F. Flint. 1986. Ultrastructural localisation of oxytocin and neurophysin in the ovine corpus luteum. Cell Tiss. Res. 243:129-135.

Ursely, J. and P.Leymarie. 1979. Varying response to luteinizing hormone of two luteal cell types isolated from bovine corpus luteum. J. Endocrinol. 83:303-310.

Vane, J.R. 1971. Inhibition of prostaglandin synthesis as a mechanism for aspirin-like drugs. Nature (New Biol.) 231:232-235.

Veldhuis, J.D. and L.M. Demers. 1986. An inhibitory role for the protein kinase C pathway in ovarian steroidogenesis. Biochem. J . 239:505-511.

Veldhuis, J.D. and L.M. Demers with the technical assistance of P. Azimi, D. Juchter and J. Garmey. 1987. Activation of protein kinase C is coupled to prostaglandin $F_{2\alpha}$ synthesis in the ovary: studies in cultured swine granulosa cells. Mol. Cell. Endocrinol. 49:249-254.

Virutamasen, P., K.H. Wright and E.E. Wallach. 1972. Effects of prostaglandins E_2 and $F_{2\alpha}$ on ovarian contractility in the rabbit. Fertil. Steril. 23:675-682.

Walles, B., L. Edvinsson, B. Falck, Ch. Owman, N.-O. Sjöberg and K.-G. Svensson. 1975a. Evidence for a neuromuscular mechanism involved in the contractility of the ovarian follicular wall: fluorescence and electron microscopy and effects of tyramine on follicle strips. Biol. Reprod. 12:239-248.

Walles, B., L. Edvinsson, C. Owman, N.-O. Sjöberg and K.-G. Svensson. 1975b. Mechanical response in the wall of ovarian follicles mediated by adrenergic receptors. J. Pharmacol. Exp. Therap. 193:460-473.

Walters, D.L., D. Schams, B. Bullerman and E. Schallenberger. 1983. Pulsatile secretion of gonadotrophins, ovarian steroids and ovarian oxytocin during luteolysis in the cow. Biol. Reprod. 28(Suppl. 1):142.

Wathes, D.C., S.E.F. Guldenaar, R.W. Swann, R. Webb, D.G. Porter and B.T. Pickering. 1986. A combined radioimmunoassay and immunocytochemical study of ovarian oxytocin production during the periovulatory period in the ewe. J. Reprod. Fertil. 78:167-183.

Wathes, D.C. and R.W. Swann. 1982. Is oxytocin an ovarian hormone? Nature 297:225-227.

Wathes, D.C., R.W. Swann, S.D. Birkett, D.G. Porter and B.T. Pickering. 1983. Characterization of oxytocin, vasopressin and neurophysin from the bovine corpus luteum. Endocrinology 113:693-698.

Wathes, D.C., R.W. Swann and B.T. Pickering. 1984. Variations in oxytocin, vasopressin and neurophysin concentrations in the bovine ovary during the oestrous cycle and pregnancy. J. Reprod. Fertil. 71:551-557.

Watkins, W.B., V.J. Choy, I.M. Chaiken and J. Spies. 1986. Isolation and sequence analysis of oxytocin from the sheep corpus luteum. Neuropeptides 7:87-95.

Watkins, W.B., L.T. Moore, A.J. Peterson and H.R. Tervit. 1984. Possible role for ovarian prostaglandin $F_{2\alpha}$ in stimulating luteal oxytocin release in ewes at luteolysis. J. Ster. Biochem. 20:1508.

Watson, J. and P.M. Wrigglesworth. 1975. Progesterone synthesis by pig corpus luteum tissue during superfusion. Biochem. J. 150:301-304.

Webb, R. and B.G. England. 1982. Relationship between LH receptor concentrations in thecal and granulosa cells and in-vivo and in-vitro steroid secretion by ovine follicles during the preovulatory period. J. Reprod. Fertil. 66:169-180.

Webb, R., M.D. Mitchell, J. Falconer and J.S. Robinson. 1981. Temporal relationships between peripheral plasma concentrations of oxytocin, progesterone and 13,14-dihydro-keto prostaglandin $F_{2\alpha}$ during the oestrous cycle and early pregnancy in the ewe. Prostaglandins 22:443-454.

Weiner, S., K.H. Wright and E.E. Wallach. 1975. Studies on the function of the denervated rabbit ovary: human chorionic gonadotropin-induced ovulation. Fertil. Steril. 26:363-368.

Weston, P.G. and J.E. Hixon. 1980. Effects of in vivo prostaglandin $F_{2\alpha}$ administration on in vitro progesterone synthesis by bovine corpora lutea. Biol. Reprod. 22:259-268.

Wiltbank, J.N. and L.E. Casida. 1956. Alteration of ovarian activity by hysterectomy. J. Anim. Sci. 15:134-140.

Yang, N.S.T., J.M. Marsh and W.J. LeMaire. 1973. Prostaglandin changes induced by ovulatory stimuli in rabbit Graafian follicles. The effect of indomethacin. Prostaglandins 4:395-404.

Zeleznik, A.J., A.R. Midgley, Jr. and L.E. Reichert, Jr. 1974. Granulosa cell maturation in the rat: increased binding of human chorionic gonadotropin following treatment with follicle-stimulating hormone *in vivo*. Endocrinology 95:818-825.

Ziecik, A., H.J. Shaw and A.P.F. Flint. 1980. Luteal LH receptors during the oestrus cycle and early pregnancy in the pig. J. Reprod. Fertil. 60:129-137.

Zor, U., S.A. Lamprecht, Z. Misulovin, Y. Koch and H.R. Lindner. 1976. Refractoriness of ovarian adenylate cyclase to continued hormonal stimulation. Biochim. Biophys. Acta 428:761-765.