Basic Mechanisms of Ovarian Endocrine Function
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This review outlines the current understanding of ovarian endocrine development and regulation with both physiological and biochemical background to provide a framework applicable to problems concerning environmental agents and ovarian endocrine function. Two approaches are used. First, the endocrine regulation of follicle development and corpus luteum function is considered in the classical sense, i.e., viewing these structures as gonadotropin-responsive units undergoing a programmed sequence of development and differentiation. Secondly, a relatively new area of ovarian physiology concerned with intra-ovarian regulation is explored, since this area holds potential for exploration of the direct effects of toxicological or environmental agents upon gonadal endocrine cells.

Follicle Development
Gonadotropin-Independent and Dependent Stages

The stages of follicular maturation have been classified in a variety of ways by several different investigators, depending upon the degree of detail necessary (1, 2). For purposes of this discussion, distinction need only be made between the following: (1) the primordial follicle stage, characterized by an immature ovum surrounded by a single layer of flattened or disc-shaped granulosa cells; (2) the primary follicle stage, characterized by a transition in granulosa cell morphology to the cuboidal form followed by an increase in the number of granulosa cell layers (In the rat, oocyte growth is complete by the four-layer granulosa cell stage); and (3) the antral or vesicular follicle stage characterized by the formation of a single fluid-filled antral cavity. After formation of the antrum further follicular growth occurs consisting mainly of enlargement of the follicular cavity culminating in the preovulatory or Graafian follicle.

Follicle development in most species, with the notable exception of the human (3), is thought to be independent of gonadotropic support up to the four-layer granulosa cell stage of the primary follicle (/). In the light of current knowledge, the acquisition by the follicle of a functional gonadotropin receptor population provides the distinction between gonadotropin-dependent or -independent stages of development. In the mouse, ovarian response to follicle stimulating hormone (FSH) or luteinizing hormone (LH) in terms of increased protein synthesis and adenylyl cyclase activity, respectively, is demonstrable during the second week of life implying that functional gonadotropin receptors are present at this early stage (4, 5). At 14 days of age, mouse oocytes are surrounded by three or more layers of follicle cells (4). In the rat, ovarian protein kinase activity is responsive to FSH or LH at 5 days of age (6). In contrast, granulosa cells of the 24-day-old immature rat have been reported to be devoid of LH receptors (7) and do not respond to LH in vitro with increased progesterone production (8). At this time the predominant ovarian structures consist of primary or pre-antral follicles. FSH and LH levels in the rat decline between 15 and 24 days of age (9), and presumably this leads, by mechanisms not understood, to the disappearance of the LH receptor population.

These circumstances in the rat have been turned to remarkable advantage in the development of a model system in which it has been possible to demonstrate that the gonadotropin-dependent developmental sequence of follicular development and luteal formation has as its basis a time- and endocrine-dependent progression of granulosa cell

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receptors for FSH, LH, and prolactin (PRL). In an important series of studies, Richards and Midgley (10) and Richards et al. (11) demonstrated that administration of estrogen and FSH to the 24-day-old hypophysectomized rat leads to a marked increase in the granulosa cell FSH receptor population; the action of estrogen and FSH in time also leads to the appearance of the LH receptor population; the process of luteinization subsequently leads to the appearance of the PRL receptor population. PRL is also present in follicular fluid, but its role in granulosa cell function is not understood at this time (12).

**Cellular and Enzymatic Basis of Steroid Secretion**

Reproductive physiologists have long been interested in determining the cellular origin of the principal steroidal products of the ovary. Incubation studies with luteal and interstitial slices definitively established that these compartments preferentially synthesized progestin and androgen, respectively, from labeled acetate (13). Similar techniques were not applicable, however, to the follicular compartment because of the close apposition of thecal and granulosa elements. The cellular basis of follicular estrogen production has thus enjoyed an interesting recent history beginning with the experiments of Falck in 1959 (14). He transplanted cell types of various ovarian compartments, singly and in certain combinations, to the anterior chamber of the eye of ovariectomized rats. Using bits of vaginal epithelium placed alongside the ovarian cell transplants as a biomarker, he obtained evidence of estrogen production only from combinations of granulosa and thecal elements. The consensus of subsequent studies with labeled precursors both in vivo and in vitro is that the thecal cells possess all of the requisite enzymatic assemblies necessary to convert cholesterol to pregnenolone, progesterone, testosterone, and estradiol. The granulosa cells, on the other hand, are extremely deficient in “desmolase,” the enzyme function which results in the conversion of C21 to C19 steroids; thus, they secrete negligible quantities of androgen. They are well equipped, however, to utilize androgen as a substrate, forming estrogen via the “aromatase” system. Moon et al. (15) and Dorrington et al. (16) have contributed an important addition to these observations by demonstrating that conversion of androgen to estrogen in the granulosa cell is under specific regulation by FSH. This marks only the second established gonadotropin-dependent rate-limiting control point in sex steroid biosynthesis subsequent to cholesterol formation, the first being the LH-dependent 20α-hydroxylation of cholesterol prior to cholesterol conversion to pregnenolone (17). For a comprehensive review of gonadal estrogen biosynthesis, the reader is referred to the recent review by Armstrong and Dorrington (18).

**Endocrine Regulation of Corpus Luteum Function**

Since several reviews of the corpus luteum (CL) including its cytology, biochemistry, and comparative aspects in addition to its endocrine regulation are available (19–23), only the general features of luteal maintenance and regression will be discussed here.

**Luteotropic Control**

In contrast to the relative uniformity in the endocrine control of follicular development and ovulation, the processes of luteal maintenance and regression show considerable species diversity. Although the life span of the corpus luteum during non-pregnant cycles varies considerably among species, it is generally true that function is maintained by a low steady-state level of circulating gonadotropin, LH in particular. LH is the predominant luteotropin in the nonpregnant woman (24); LH and prolactin maximize luteal progesterone secretion in the sheep (25) and probably also the rat (26); the hamster CL is dependent upon a luteotropic complex consisting of FSH, LH, and prolactin (27, 28). Even though serum LH and steroid levels do not indicate a dynamic “push-pull” relationship during the luteal phase, the continued presence of LH or LH and PRL is necessary for normal progesterone synthesis in both primates (23, 24) and nonprimates (26, 29).

The question of maintenance is not a simple one, however. Distinction has been made between different types of luteotropic activity based upon the primary activity of the luteotropin, i.e., steroidogenic vs. structural effects. LH has long been known to stimulate steroidogenesis by luteal tissue acutely or chronically, in vitro (13) or in vivo (30). It is equally well known that prolactin is absolutely critical for maintenance of the CL in the rat, for example (30), but it is not steroidogenic in the acute sense in vivo (31) or in vitro (32). Yet PRL may somehow synergize with LH to maximize luteal progesterone secretion in vivo (25). Thus, the extent to which prolactin is luteotropic strictly in the structural vs. the steroidogenic sense and the mechanism(s) of its action are unclear (33). Some
studies suggest its steroidogenic control might be accomplished via regulation of the availability of cholesterol stores (34).

The mechanism of LH action upon luteal steroidogenesis has been actively investigated for several years and has been the subject of several authoritative reviews (13, 35–37). In common with several other polypeptide hormones, its initial action involves binding to a plasma membrane receptor, activation of adenyl cyclase and generation of intracellular cyclic AMP. The possible role of cyclic AMP and of cyclic AMP-dependent protein kinase has been reviewed in depth very recently and is highly recommended to the interested reader (38). Briefly, evidence for the role of cyclic AMP action in steroidogenesis is discussed in terms of several intracellular control points: (1) cofactor availability, (2) substrate availability, (3) cholesterol transport, (4) cholesterol side-chain cleavage activity, and (5) mitochondrial efflux of steroid substrate. The role of protein synthesis in LH-stimulated steroidogenesis is also addressed. In addition to the model systems to be discussed later, it would seem that the in vitro techniques utilized in the studies just mentioned would also be applicable to studies of the mechanism(s) by which environmental agents influence gonadal steroidogenesis.

Luteolytic Control

In general, the CL of those species which exhibit hysterectomy-induced prolongation also exhibit prostaglandin F2α-induced luteolysis; furthermore, luteal regression is accomplished experimentally in the presence of elevated LH or prolactin levels (39, 40). Therefore, the lytic stimulus in these species is thought of as being extrinsic to the ovary and functionally dominant to steady-state trophic stimuli.

The mechanism(s) underlying prostaglandin-induced luteolysis is incompletely understood at this time. Detailed attention has been given for the past several years to the suggestion by Pharriss (41) that PGF2α-induced luteolysis resulted from selective ovarian veno-constrictive action. Experimental results have been divided. Studies in the ewe by Niswender et al. (42) and Nett et al. (43) indicate that PGF2α administration during the mid-luteal phase of the cycle results in hemodynamic changes in the ovary bearing the corpus luteum which are similar to those seen during normal luteolysis. On the other hand, several studies failed to support the hypothesis: (1) mechanical reduction of blood flow to the sheep ovary during the mid-luteal phase of the cycle did not induce luteolysis (44); (2) experiments in the rabbit indicated PGF2α did not consistently change the vascular resistance of the corpora lutea (45); and (3) PGF2α induced regression of corpora lutea transplanted to the rabbit kidney capsule, thus ruling out the possibility of hemodynamic effects upon the ovarian vein (46). In spite of these negative findings, however, the possible involvement of vascular mechanisms in luteolysis still cannot be definitively excluded.

The biochemical mechanisms of luteolysis are also only incompletely understood. Some of the current studies which are germane to this area are: the effects of PGF2α upon LH receptor mechanisms (47), the role of estrogen in prostaglandin-induced luteolysis (48, 49) as well as the direct effects of estradiol upon luteal cell progesterone secretion (50), and the role of lysosomes in luteal regression (51).

In those species in which hysterectomy does not extend the luteal phase, notably the human and the rhesus monkey (23, 52), evidence for prostaglandin-induced luteolysis is likewise meager (53). Considerable uncertainty still exists in primates with regard to the role of prostaglandins in luteal function. As examples, one need only cite observations such as the following which are conceptually difficult to relate at this point in time: (1) exogenous PGF2α does not induce luteolysis in the majority of cases (53); (2) membrane preparations of human luteal tissue contain binding sites for prostaglandin F2α (54); and (3) the ovary itself may produce sufficient prostaglandin to saturate available receptor sites (55, 56). Since evidence such as this cautions against acceptance of the concept of a generalized and dominant prostaglandin-induced lytic mechanism, a more passive type of luteolytic control has been postulated, i.e., the withdrawal of luteotropic support (57). Such a concept could include local cellular mechanisms which decreased gonadotropin receptor number or function. It is only fair to note, however, that such a mechanism has not yet been demonstrated experimentally.

Intra-ovarian Regulation and the Direct Study of Ovarian Endocrine Cells

In addition to gonadotropin regulation of ovarian endocrine function, studies in vitro with isolated intact ovarian cells are re-emphasizing the possibility of an additional level of control modulated by the ovarian steroids themselves. Studies with primary cultures of ovarian granulosa cells have provided, for example, direct demonstrations of the stimulation of progesterone secretion by dihydrotestosterone (DHT) (Fig. 1) or DHT–FSH interactions (58–60), and of estradiol suppression of progesterone secretion (58). The latter finding has also been noted in isolated luteal cells (50).
Direct Study of Ovarian Cell Types

With the advent of a variety of techniques for cell dispersion, cell separation, and in vitro culture, it is now possible to study directly specific isolated ovarian cell types. It is obvious that such an approach should not become an end in itself but should contribute to a more comprehensive understanding of ovarian physiology in the broad sense of the term. This goal depends in part, however, upon a working knowledge of the various individual cellular components. Since this aspect is in its infancy, it will be reasonable to expect expanded efforts in the study of isolated ovarian cell types at the basic research level. The feasibility for application of such systems to problems concerning the environment and reproduction remains to be determined however. Their value to questions concerned with biochemical mechanisms of action is obvious, but the scope of usefulness may be broader than this. For example, since granulosa cells are the only other ovarian cell type sharing the follicular antral compartment with the ovum and are readily studied in vitro, is it possible that isolated granulosa cell systems might serve a role in studies of the detection, metabolism, or detoxification of documented or potential germ cell mutagens?

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Intra-ovarian Regulation

The observations cited above are not the first examples of intra-ovarian effects elicited by steroids. In 1940, it was shown that either diethylstilbestrol (DES) or estradiol-17β markedly stimulated granulosa cell proliferation and also increased ovarian responsiveness to gonadotropins in the immature hypophysectomized rat (61, 62). The effects of estrogen and progesterone upon ovum maturation in vitro provide another possible example(s) of steroid mediated intra-ovarian regulation (63).

Nonsteroidal compounds are also part of the regulatory process at both the inter- and intracellular levels. Since ovulation can be blocked in rabbits by prostaglandin synthesis inhibitors such as indomethacin (64), prostaglandin action is thought to be one of the components responsible for follicle rupture. Other nonsteroidal compounds, as yet unidentified, with the following activities and sites of origin have been reported: a luteinization inhibitor(s) contained in follicular fluid (65), a meiosis-inhibiting factor(s), also contained in follicular fluid (66); and an inhibitor present in luteal tissue which inhibits 125I-human chorionic gonadotropin binding (67). It goes without saying that the potential capability for pinpointing the biochemical effects of environmental agents increases significantly as new regulatory possibilities such as those mentioned above become better understood.
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