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Current Status of the Hypothesis That Mammalian Ovulation Is Comparable to an Inflammatory Reaction

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ABSTRACT

This presentation reviews current information on the events that lead to rupture of an ovarian follicle. It contains a summary of the morphological changes that occur at the apex of a follicle wall during ovulation. Existing information shows that the tenacious connective tissue layers of the tunica albuginea and theca externa must be weakened before the follicle wall can dissociate and break open under the force of a modest intrafollicular pressure. These changes are probably dependent on transformation of quiescent thecal fibroblasts into proliferating cells in a manner that is characteristic of tissue responses to inflammatory reactions. The metabolic factors that initiate transformation of the fibroblasts are uncertain, but they are probably generated by gonadotropin-induced changes in the theca interna and granulosa of a follicle as these layers begin to luteinize during the ovulatory process.

INTRODUCTION

Mammalian ovulation is a distinct biologic phenomenon that requires the rupture of healthy tissue at the surface of the ovary. The first major review of literature on ovulation was written by Carl Hartman in 1932 [1]. This early account pointed out that ovulation is a "sine qua non" of the reproductive process—an indispensable event for propagation of a species. Hartman went on to state that "cyclic sexual phenomena in the maternal organism are most conveniently separated into those occurring before and those occurring after ovulation." Accordingly, the topic of this symposium has been divided into folliculogenesis, ovulation, and luteinization.

Thirty years after Hartman's review, Asdell summarized the principal theories on the mechanism of ovulation that were under consideration in 1962 [2]. At that time it was generally assumed that mammalian follicles rupture as a consequence of increasing intrafollicular pressure. It was also thought that contraction of smooth muscle tissue in the ovarian stroma promoted the increase in pressure. The present review is an overview of the knowledge that has been gained since 1962. The initial section is an examination of some of the morphological changes that occur in ovulatory follicles. The central section is an assessment of the current status of the theory that the events of ovulation are comparable to an inflammatory reaction. The final section presents the conclusion that inflammatory-like changes first occur in the theca interna and granulosa layers of the follicle in response to gonadotropic stimulation of the luteinization process. The production of as yet undetermined factors within these two innermost layers causes activation and proliferation of the fibroblasts in the collagenous tissue that surrounds mature follicles. During this mobilization of thecal fibroblasts, the follicle wall becomes precariously weaker and eventually ruptures under the force of a modest, but adequate, intrafollicular pressure.

FUNCTIONAL ANATOMY AND OTHER BACKGROUND INFORMATION

At the apex of a follicle, where a stigma forms and the follicle ruptures, there are five different layers of cells [3]. The outermost layer is the surface epithelium, a single-cell layer of cuboidal epithelial cells. These cells are characterized by large, polymorphous nuclei and a number of dense granules in their cytoplasm. During the 1970s, some consideration was given to the possibility that these dense granules might be the source of proteolytic enzymes that degrade the follicle wall and cause it to rupture [4]. However, it now appears that the granules are not involved in ovulation, and their composition and function remain uncertain [3]. The second layer is the tunica albuginea, consisting of fibroblasts and collagen that form a tenacious sheath around the entire ovary and delineate its integrity. This tunic is usually about 5–7 cells deep. The extracellular collagen fibers, which these cells produce, are distributed as a circumfluent matrix around the entire ovary. The third layer is the theca externa, the follicles' own capsule of collagenous connective tissue. This layer also contains fibroblasts about 5–7 cells deep. At the apex of a mature follicle, in the area where the theca externa is contiguous with the tunica albuginea, the two layers of similar tissue mesh in a manner that makes it difficult to distinguish them from one another. When thin sections are cut on a plane that displays a cross-sectional view of the full-wall thickness, the fibroblasts appear to be spindle-shaped. When the sections are cut transversely (on a plane parallel to the surface of the ovary), however, the third dimension reveals an ovoid shape that confirms the platter-like configuration characteristic of fibroblasts within layers of thecal tissue [5]. Also, transverse sectioning cuts the collagen fibers in a more conspicuous longitudinal plane and reveals their formation from the fi-

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broblasts. The fourth layer is the theca interna, just inside the theca externa. This thin layer of steroid-secreting cells is about two cells deep. The cytoplasm of these cells contains numerous mitochondria and lipid droplets dispersed within smooth endoplasmic reticulum. This metabolically active layer receives most of the capillary supply to the follicle. The fifth layer is the stratum granulosum, about 5–7 cells deep except at the random site where the cells form a pedestal, called the cumulus oophorus, which supports the oocyte. The outermost ring of granulosa cells is attached to a basement membrane that separates this layer from the theca interna. The capillaries of the theca interna cannot penetrate this basal lamina or enter the avascular granulosa. A unique feature of the granulosa is that an elaborate network of gap junctions unites the cells of this layer into a functional syncytium. The nutritional and electrical coupling provided by this syncytium might permit the granulosa to influence oocyte metabolism or vice versa.

The duration of the ovulatory process varies among different species of mammals. It may be as short as 10 h in an animal like the rabbit or as long as 40 h in a human being. During the final hour before a follicle actually ruptures, several distinct changes are visible in the ultrastructure of the follicle wall [3, 5, 6]. It is especially apparent that the fibroblasts of the tunica albuginea and theca externa have changed from a quiescent, resting state to an active, proliferating state. These connective tissue cells become much more elongate, and cytoplasmic processes extend far from the central mass of their cytoplasm. Also, the fibroblasts begin to dissociate from one another; the tunica albuginea and theca externa at the apex of the follicle take on the appearance of a looser, less tenacious connective tissue. The degradative events within this tissue spread up into the surface epithelium, and the cuboidal cells of this outer layer become vacuolate and necrotic. Toward the interior of the follicle, the cells of the theca interna remain relatively unchanged. Most of the large capillaries in this thecal layer continue to be patent and flowing, but a few have coagulated blood, perhaps accounting for the occasional appearance of petechia on the surface of follicles that are about to rupture. Also, the blood vessels appear to contain more white blood cells, but there is negligible evidence that these leukocytes migrate out of the vascular compartment into the surrounding tissues. It is possible that an ovulatory increase in vascular permeability permits exudation of serum components into the extracellular matrix and that as yet unidentified elements of the serum serve to activate the thecal fibroblasts. Lastly, at the interior of the follicle wall, the granulosa cells begin to exhibit lipid droplets in their cytoplasm during the final hours before rupture. This accumulation of lipid probably reflects the increase in progesterone synthesis that occurs during the latter part of the ovulatory process.

During the final minutes before a follicle actually ruptures, there are marked changes at the apex of the follicle wall [3, 6]. The fibroblasts become much more dissociated, and the apex balloons out to form the so-called stigma. Usually, the surface epithelial cells slough off the ovary at the site where rupture normally occurs. In addition, as the thecal tissue dissociates and the stigma forms, the cells of the theca interna and granulosa layers break apart and retract to the base of the stigma. Thus, the last remaining tissue at the site of pending rupture is highly degraded collagenous tissue. Just before the wall breaks, it usually narrows to no more than 20% of its preovulatory thickness [6].

Dissociation of the follicle wall and eventual rupture are dependent on the hydrostatic pressure within the follicular antrum. This modest pressure of about 15–20 mm Hg does not change during the ovulatory process [7], but it nevertheless appears to be important for ovulation. The source of the intrafollicular pressure is the capillary hydrostatic pressure within the vasculature of the theca interna. Therefore, any conditions that alter systemic blood pressure will also influence the intrafollicular pressure.

INFLAMMATORY-LIKE CHANGES DURING OVULATION

The hypothesis that ovulatory follicles become inflamed is now supported by a variety of experimental evidence [3, 8]. It is especially clear that any potent nonsteroidal anti-inflammatory agent (such as indomethacin) will inhibit ovulation if the drug is administered during the first 80% of the ovulatory process. In contrast, steroidal anti-inflammatory drugs (such as dexamethasone) do not inhibit ovulation, but such steroids usually have an effect on chronic inflammatory conditions rather than on acute inflammatory processes.

Most of the work on inflammation and ovulation during the past 12 yr has been conducted on rat ovarian tissue. The immature rat model has been especially useful in these efforts. Ovulation is induced in this model by first weaning the immature rats at about 22 days of age. Folliculogenesis is induced 24 h later by a single s.c. injection of eCG. A dose of 4 IU eCG will stimulate a normal number of follicles (i.e., 10–15) to develop and mature in a pair of ovaries, whereas a dose of 10 IU eCG will promote the development of 60–70 follicles and results in superovulation. Forty-eight hours after the animals are primed with eCG, the ovulatory process is initiated by a single injection of hCG. Within 10–12 h after the hCG treatment, ova begin to appear in the oviducts and ovulation is complete by 16–20 h after administration of hCG.

Historically, inflammation has been defined as "redness and swelling, with heat and pain—rubor et tumor cum calore et dolor" [9]. This definition dates back 2000 years to the writings of Cornelius Celsus (not to be confused with Celsius of temperature repute) in his description of a typical response of flesh to a microbial infection. The redness and swelling are the result of a local increase in blood volume and edema due to vasodilatation and increased vas-

cular permeability. In rat ovaries, a similar hyperemic response begins within a few hours after the ovulatory process has been initiated by gonadotropin, and it persists to the time of follicular rupture [10]. Thus, the ovulatory process is also characterized by this cardinal feature of an acute inflammatory reaction.

The biochemical events of an inflammatory reaction are much more complex and much less well understood. The so-called "inflammation cascade" consists of a number of agents that mediate and moderate inflammatory processes by direct and indirect actions on the affected tissue [3, 8, 11]. Collectively the biochemical events cause an increase in vasodilatation, hyperemia, exudation, edema, collagenolysis, cell proliferation, tissue remodeling, and other common changes in inflamed tissue.

During the past decade, a number of the agents commonly associated with the inflammation cascade have been studied in ovulatory tissue [3, 8]. Vasoactive agents like bradykinin, histamine, and platelet-activating factor (PAF) have all been measured in ovaries stimulated with ovulatory doses of gonadotropin. Kinin formation begins to increase in rat ovaries during the first several hours of the ovulatory process. Eventually, by the time follicles begin to rupture, this agent is elevated about 10-fold. In inflamed tissues, bradykinin and other such kinins are well known for their promotion of vasodilatation, and this is their presumed role in the ovulatory process. In contrast, the measurable levels of ovarian histamine decrease during the ovulatory process [3, 8]. This decline is probably the consequence of degranulation of histamine from the mast cells along the ovarian blood vessels and subsequent dissipation of this vasoactive agent from the local area. The released histamine is thought to contribute to the vasodilatation that occurs in ovulatory follicles. Similarly to histamine, ovarian PAF decreases markedly in its measurable amount during the first several hours of the ovulatory process, and this membrane phospholipid remains relatively low during the course of the ovulatory process. Apparently, during gonadotropin-induced changes in membrane signal transduction processes and activation of the arachidonate cascade, some unknown events reduce the measurable amount of PAF. In any case, this local decline in measurable PAF is characteristic of inflamed tissues, and the release of this agent should promote the same kind of vascular changes as are induced by bradykinin and histamine.

Inflammatory reactions invariably include activation of the arachidonate cascade by phospholipases and subsequent action of prostaglandin synthetases and lipoxygenases on arachidonic acid [3, 12]. The prostanoids, in particular, have been given considerable attention during the past two decades as potential mediators of ovulation. Prostaglandins (PG) of the E and F type begin to increase in ovarian follicles during the first several hours of the ovulatory process. They reach a peak at about the time the follicles begin to rupture, and shortly thereafter their ovarian levels decline toward preovulatory values. The amount of PGE at any given time during the ovulatory process is usually about twice that of ovarian PGF. The specific roles of these prostanoids in ovulation are not clear. In fact, it is not even clear whether they function to mediate, or to moderate, the ovulatory process. In inflammatory reactions, there are reports that PGs may have both pro- and anti-inflammatory roles [13]. Thus, it remains uncertain whether the ovulatory rise in prostanoids serves to promote the degradative events that lead to follicular rupture or whether these metabolites of arachidonic acid are a response to the inflammatory-like changes in the follicle.

It is important to keep in mind that in addition to the PG pathway from arachidonic acid, there are a number of other eicosanoid pathways that are regulated by a family of lipoxygenase enzymes [3]. These enzymes lead to the formation of leukotrienes, lipoxins, and probably other bioactive agents that influence inflammatory reactions. Leukotrienes, which are produced as a result of 5-lipoxygenase activity, increase modestly in rat ovaries during the first several hours of the ovulatory process; but they decline to preovulatory levels during the 6 h preceding follicular rupture. There is substantially more leukotriene C_4 than leukotriene B_4 in the ovary. In other models of inflammation, peptidic leukotriene C_4 might function to stimulate motility and proliferation of cells, while leukotriene B_4 has usually been associated with the attraction of white blood cells into inflamed areas. The specific roles of these agents in ovulation have not been clearly defined. In contrast to the leukotrienes, products of 12- and 15-lipoxygenase activities begin to increase significantly in rat ovaries at about 4–6 h into the ovulatory process. (The activities of these enzymes are usually estimated by measuring tissue levels of 12- and 15-hydroxyeicosatetraenoic acids (HETE), since these acids are relatively stable products of the respective enzymes.) Both of these eicosanoids reach a peak in the rat ovary at about the time when follicles begin to rupture. The function of 12-HETE is unknown; but 15-HETE is associated with the formation of lipoxins, which may cause migration of endothelial cells and induce angiogenesis [14]. Therefore, lipoxins could promote the formation of new capillaries in the developing lutein tissue in ovulatory follicles.

The information reviewed above makes it clear that a number of eicosanoids besides the prostanoids could be involved in the ovulatory process. In view of this possibility, studies have been conducted to compare the ovarian levels of PGs with the levels of certain products of the lipoxygenase pathway of arachidonate metabolism in rats and rabbits treated with ovulation-inhibiting doses of indomethacin [3]. The results show that low doses of indomethacin that strongly inhibit ovarian PG synthesis have no effect on ovulation rate or on 15-lipoxygenase activity and 15-HETE synthesis. On the other hand, intermediate and high doses of indomethacin inhibit both ovulation rate and ovarian 15-HETE formation. Statistical analyses reveal that the ovula-

tion rate is more closely correlated with ovarian 15-HETE levels than with ovarian PG levels. Collectively, these studies provide several important pieces of information. First, it is now clear that indomethacin is not as specific an inhibitor of prostanoid synthesis as previously thought. Secondly, it appears that lipoxygenase products of arachidonate metabolism could have significant roles in ovulation; their specific functions deserve greater attention in future studies. Thirdly, while prostanoids might contribute in some way to the mechanism of ovulation, their specific roles remain uncertain and their designation as mediators of the process probably should no longer be taken for granted.

In view of this information, there has been a recent reassessment of the capacity of exogenous prostanoids to overcome the inhibitory action of indomethacin on ovulation in the rat [15]. When equivalent groups of animals were given a constant dose of 1.0 mg of indomethacin to inhibit ovulation, in no instance did large doses of exogenous PGE, PGF, or the two prostanoids together bring about any recovery of the ovulation rate. Instead, to the contrary, in parallel groups of animals that were not treated with indomethacin and that would have normally ovulated, the ovulation rate was actually inhibited (by about 50%) when the animals were given doses of PGE alone or in combination with PGF. Thus, the significance of prostanoids in ovulation remains uncertain, and it is yet to be determined whether these agents exert a pro- or an anti-inflammatory action during ovulation.

This information suggests that indomethacin exerts its antiovulatory effect by inhibiting the synthesis of one or more ovarian eicosanoids. However, even if this assessment is accurate, the specific roles of the eicosanoids in ovulation have not been determined. Eicosanoids do not appear to mediate the ovulatory increase in ovarian progesterone synthesis, because luteinization can proceed unabated in animals that have been treated with indomethacin [3]. Also, there is evidence that indomethacin does not interfere significantly with ovarian collagenolytic activity during ovulation [16]. Furthermore, indomethacin does not block the initial stages of connective tissue degradation at the apex of ovulatory follicles [17]. Thus, the inhibitory action of this nonsteroidal anti-inflammatory agent remains uncertain. One possibility is that it might impair full motility of the thecal fibroblasts and thereby critically reduce the dissociation of cells within the tunica albuginea and theca externa at the apex of a follicle. Another possibility is that indomethacin might alter the ovarian vasculature in some way that reduces blood pressure and attenuates the hydrostatic force within the follicle that is essential for rupture.

The increase in eicosanoids during ovulation presumably mediates proteolytic degradation of the thecal connective tissue in the follicle wall, but the specific nature of this mediation has not been deciphered. Three types of proteolytic activity that have been associated with inflammatory reactions have been assessed for their possible involvement in the ovulatory process. These are glandular kallikrein, tissue-type plasminogen activator, and interstitial collagenase [3, 8]. Kallikrein activity increases about 5-fold in rat ovaries during ovulation. This serine protease is known for its ability to convert kininogens into kinins. In addition, it may function to hydrolyze procollagenase into its active form. Plasminogen activator increases about 10-fold during ovulation, but the importance of this serine protease for follicular rupture has not been firmly established. It is usually associated with plasmin activity and fibrinolysis, and, like kallikrein, it has been implicated as an activator of procollagenase. Interstitial collagenase has been considered to be the agent most likely to cause degradation of thecal connective tissue in the follicle wall ever since the demonstration, almost 30 yr ago, that injection of this metalloprotease into the antrum of rabbit follicles could induce follicular rupture within 10 min [18]. Since that time, a variety of direct and indirect evidence indicates that the collagenous tissue in the follicle wall is degraded during ovulation. In addition, there is evidence that extracts of ovulatory tissue can digest native collagen [19]. Still, the precise enzymes that cause ovulation have not been firmly established.

The cellular origin of the proteolytic activity that degrades the follicle wall is not known, either. In other tissues that become inflamed, the enzymes causing local degradation of the insulted area are usually produced by fibroblasts [3]. Therefore, the most likely source for such enzymes in the follicle are the thecal fibroblasts of the tunica albuginea and theca externa. This seems especially true in light of the demonstration by ultrastructural studies that the follicular fibroblasts transform from a quiescent state to a proliferative state during ovulation. In order for these connective tissue cells to become motile within the collagenous layers of the follicle, it would seem imperative that they express enzymes capable of softening the extracellular matrix that confines them to the follicle wall. Also, since fibroblasts move in an ameboid fashion, it would seem especially necessary for the cytoplasmic processes projecting from the central mass of their cytoplasm to have the capacity to soften the extracellular matrix through which they expand. In this regard, there are reports that unique multivesicular structures exist at the tips of the cytoplasmic processes of follicular fibroblasts [3, 20]. These structures might contain proteases, or protease-activating agents, that regulate the tenacity of the extracellular collagen and ground substance through which fibroblasts migrate. In any event, it seems rather certain that degradation of the thecal connective tissue and loss of tensile strength of the follicle wall are prerequisites for rupture of a follicle.

A number of other factors that have been implicated in inflammation have just begun to be evaluated as potential mediators of the ovulatory process. These include interleukins, tumor necrosis factor, and the various mitogenic growth factors such as fibroblast growth factor and epidermal growth factor [3, 11]. In addition, increasing attention has been given

to leukocytes as a possible source of the degradative enzymes that weaken the follicle wall. However, although such blood cells appear to increase in the vascular compartment of ovulatory follicles, there is no evidence that they migrate into the connective tissue layers in a position where they could degrade the follicle wall. Therefore, it is doubtful that leukocytes have a central role in ovulation. Other well-known mediators of inflammatory reactions that have not yet received attention in experiments on ovulation include Hageman factor and anaphylatoxins such as C3, C4, and C5 of the complement system. Also, it might be worthwhile to assess the potential role of free radicals such as the superoxides in ovulation.

OVARIAN STEROIDOGENESIS AND OVULATION

Steroids are not usually classified as mediators of inflammatory reactions, but it is firmly established that ovarian steroidogenic activity changes markedly during ovulation; therefore steroids must also be considered in any discussion about the mechanism of ovulation. At the beginning of the ovulatory process, estradiol synthesis is relatively high while progesterone synthesis is negligible. During the first half of the ovulatory process, however, estradiol synthesis drops to nil while progesterone synthesis increases many times over [3]. If synthesis of this progestin is blocked by 3β-hydroxysteroid dehydrogenase inhibitors like epostane or aminoglutethimide, the ovulation rate is inhibited very significantly. Furthermore, the blockage of ovulation by these inhibitory drugs can be overcome if the experimental animals are treated with an exogenous dose of progesterone. Together, these data provide strong evidence that progesterone is important in ovulation; but the pathway by which this steroid leads to proteolytic degradation of the follicle wall has not been determined.

One of the most distinct biochemical events during ovulation, as just mentioned, is the marked increase in follicular progesterone synthesis. Since progesterone synthesis is the principal biochemical indicator of luteal function, it is reasonable to deduce that luteinization of follicular tissue begins even before a follicle ruptures. Furthermore, there is ample evidence that follicular rupture is not a prerequisite for luteinization [3]. For example, the inhibition of ovulation by indomethacin does not block the conversion of a follicle into lutein tissue or interfere with progesterone synthesis. Thus, LH, as its name suggests, initiates luteinization with or without physical rupture of the follicle. Conversely, it does not appear that ovulation can occur unless a follicle enters the initial stages of the luteinization process and progesterone production.

Since luteinization is based on the transformation of the theca interna and stratum granulosum into steroidogenically active lutein tissue, it is reasonable to assume that the initial events of the "ovulatory process" occur in these layers of the follicle wall. It also seems likely that the theca interna and granulosa layers are producing one or more factors that diffuse out into the connective tissue layers of a follicle and act on the thecal fibroblasts to transform them from a quiescent state to motile, proliferating cells. The activation might be brought about by direct action of eicosanoids, bradykinin, growth factors, interleukin-1, tumor necrosis factor-α, or other agents generated by the inner layers of the follicle wall. An alternative, less direct pathway for activation might occur via vascular changes in the thecal capillaries and exudation of serum components into the follicular connective tissue to stimulate the fibroblasts. In any event, it would seem that a better understanding of the mechanisms that activate thecal fibroblasts is essential to a clearer understanding of the mechanism of ovulation.

CONCLUSIONS

Certain aspects of the ovulatory process resemble an acute inflammatory reaction. The inflammatory response appears to be centered within the theca interna and granulosa layers where luteinization events are located. Actual rupture of the follicle is probably dependent on the tissue remodeling that is characteristic of inflammatory reactions and includes mobilization of thecal fibroblasts and loosening of connective tissue elements in the follicle wall.

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